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# Gas Exchange by *Pinus ponderosa* in Relation to Atmospheric Pollutants

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY



AIR RESOURCES BOARD  
Research Division



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*Prepared for:*

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## Executive Summary

To assess potential impacts of pollutant exposure on plant performance, it is necessary to not only identify and quantify the various pollutant constituents, but also to determine the levels of deposition and uptake. Measures of foliage gas exchange rates are important to determining the ozone dosage received by forest trees. A common hypothesis is that plants with low gas exchange rates (low stomatal conductance) incur less ozone damage due to reduced oxidant uptake. Alternatively, plants having low gas exchange rates have reduced potential for carbon fixation and, therefore, a lesser potential for growth or damage repair.

The purpose of the study reported here was to determine the extent to which realistic exposures to acid rain and ozone pollutants, acting singly and together, influence gas exchange rates by *Pinus ponderosa* (ponderosa pine), a tree of substantial economic, ecological, and aesthetic importance in California. As secondary objectives, the extent of response to pollutants as a function of life-stage (seedling or mature tree), foliage age (current-year or one-year-old foliage) and genotype were explored.

Ponderosa pine seedlings and mature tree branches were exposed to combinations of ozone and acid rain using Branch Exposure Chambers (BECs) for a 15-month period beginning in September 1991. Acid rain treatments consisted of no rain or 17 weekly applications of 5 cm simulated rain of pH 5.1 or pH 3.0. The rainfall applications were made between January and May, 1992, to coincide with the natural rainfall season. Ozone treatments consisted of charcoal filtered air, ambient ozone or twice ambient ozone applied for 14 h per day. The ozone and acid rain exposures were applied as a complete factorial treatment structure. Ambient ozone concentration (12-h average from 0900 to 2100 h) at the study site ranged from approximately 0.01 ppm in January to approximately 0.07 ppm in July and August. Relative to the 12-h average ozone concentration for the ambient treatment, ozone concentrations for charcoal-filtered and twice ambient treatments were approximately 55 and 190 percent, respectively, when averaged over the experiment.

Measurements of mid-day photosynthesis and stomatal conductance were made on one-year-old and current-year foliage at monthly intervals from February through November. Estimates of foliar pigment concentrations were made in May and September to provide a relative measure of pollutant stress.

The only significant effect of acid rain, relative to no rain, was a slight reduction in stomatal conductance for one-year-old seedling foliage exposed to pH 5.1 or pH 3.0 rain solutions.

Relative to ambient ozone, exposure to twice ambient ozone resulted in significant reductions in photosynthesis and conductance for one-year-old mature branch foliage. This effect was most pronounced in the early summer and fall of 1992 as both stomatal conductance and photosynthesis were decreased by as much as 34 percent when exposed to twice ambient ozone.

For the study period, gas exchange rates for seedling foliage were substantially affected by exposure to twice ambient ozone and the degree of effect varied by genotype. Apparent reductions in conductance reached approximately 15 percent while reductions

in photosynthesis were as high as 35 percent. As with mature branches, the effects were more pronounced in one-year-old foliage.

Seedling foliage tended to have higher rates of stomatal conductance and similar rates of photosynthesis compared to those of mature branches for most of the season. Late season gas exchange rates of one-year-old seedling foliage dropped off in contrast with increases for one-year-old branch foliage.

Of the three seedling genotypes, half-sibs of clone 3088 were most sensitive as gas exchange rates at twice ambient ozone were decreased, relative to ambient ozone exposure, for both current-year and one-year-old foliage. Declines in gas exchange for clone 3399 were present in one-year-old foliage only. Relative to ambient ozone, twice ambient ozone exposure had no impact on gas exchange by clone 3087 for either age-class of foliage.

When measured under controlled light and temperature conditions, mature branch and seedling foliage exposed to twice ambient ozone had net photosynthesis rates that were significantly decreased relative to those for the ambient ozone treatment. The effect of twice ambient ozone was not only to alter the photosynthetic capacity of the exposed foliage, as indicated by a downward shift in the response surface, but it also resulted in change in the functional response of  $P_n$  to temperature, and to a lesser extent, light intensity.

Foliar pigment concentrations declined from May to September with the greatest decline occurring for foliage exposed to rain of pH 3.0 and twice ambient ozone concentrations. There was little statistical difference among treatments in May but by September, treatment effects were manifest as significant interactions among ozone, acid rain and genotype. In general, there were consistent declines in chlorophyll a, chlorophyll b and carotenoids under the most severe acid rain and ozone treatments. When exposed to low and moderate levels of pollutants, pigmentation responses were inconsistent among the three genotypes. The observed decreases in chlorophyll concentration, indicating potential decreases in light harvesting capacity, are consistent with the observed declines in photosynthesis rates.

From this study, the following conclusions may be drawn:

Acid rain exposure had little impact on foliar gas-exchange by seedlings or mature trees of ponderosa pine.

Long-term exposure to twice ambient ozone concentrations resulted in lower rates of photosynthesis and stomatal conductance.

The relative impact of ozone exposure on gas-exchange rates varies significantly with season.

Functional relationships describing gas-exchange response to microclimate may be altered by exposure to atmospheric pollutants.

Pollutant induced decreases in mid-day gas-exchange rates tended to be greater for mature branches than for seedlings and tended to be greater for one-year-old foliage than for current-year foliage.

Gas-exchange response of ponderosa pine to atmospheric pollutants varied substantially among three genotypes of comparable geographic origin.

**Disclaimer:**

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Air Resources Board. The mention of commercial products and their source is made for the convenience of the reader and not as an implied or actual endorsement of these products.



## I. Introduction

The impacts of air pollutants on forest ecosystems have received considerable attention worldwide as reports of regional "Forest Decline" emerged during the 1980's (Hileman 1984, Rose 1985). The primary pollutant factors have been shown to vary from one region to another and in many cases, identification of the principal factors is under debate (Rose 1985). In many instances, the primary causal agents are believed to be acid precipitation or ozone with the pollutants interacting together and with other environmental stresses (Hileman 1984, Chappelka *et al.* 1985, Reich *et al.* 1986). Although the impacts of pollutants on forests of the United States are best known for the eastern part of the country, the significant concentrations of ozone occurring in the western U.S. suggest that the health and productivity of forests in this region may also be impacted by atmospheric pollutants.

### A. Acid Precipitation

Acid precipitation has been considered a pollutant in forests of the eastern United States, but significant acid precipitation effects in the western U.S. remain largely unknown (McColl and Johnson 1983). However, acid precipitation is common and widespread in California (McColl and Johnson 1983). In the eastern U.S., where the effects of acid precipitation have been investigated in detail, the pH of the cloudwater (which is lower than that for rain) has been measured at 3.5. In California, where the effects of acid precipitation on vegetation have been minimally investigated, the pH in fog has been measured as low as 2.5 (Tomlinson 1983) and 1.69 (Jacob *et al.* 1985). Furthermore, the pH of rainwater for the Lake Tahoe region of the Sierra Nevada has been shown to have a monthly pH average as low as 4.0 (Leonard *et al.* 1981). The potential for acid precipitation effects in western forests may therefore be at least as high as that for eastern forests.

Many of the impacts on forest vegetation associated with acid precipitation are believed to be soil mediated and many studies have been undertaken to determine the effects of acid deposition on soils (Cole and Johnson 1977, Cronan *et al.* 1978, Abrahamsen 1980, Richter *et al.* 1983, DeWalle *et al.* 1985). Acid deposition may increase leaching of  $Mg^{2+}$ ,  $Ca^{2+}$ , and  $K^+$ . This loss of soil cations is accompanied by changes in nutrient cycling and forest soil nutrition. A second effect of soil acidification may be heavy metal toxicity as elements such as Al become mobilized with increasing acidity of the soil solution (Carey *et al.* 1986). However, high levels of heavy metal elements must be present before acid deposition mediated toxicity effects could occur (Abrahamsen 1984).

Direct foliar injury due to acid precipitation has been demonstrated in several studies (Wood and Bormann 1974, 1975; Evans and Curry 1979, Evans *et al.* 1977, 1978, 1981; Shriner 1977). The injury usually occurs as necrotic spots or lesions at acid precipitations of pH 3.0 or lower. Erosion of epicuticular waxes and cuticular penetration has also been reported (Schonherr 1976, Shriner 1977, Schonherr and Schmidt 1979).

The interception of wet deposition can potentially result in nutrient deficiency in leached foliage (Fairfax and Lepp 1975, Wood and Bormann 1977, Hileman 1984, Hart et al. 1986, Skeffington and Roberts 1985). While leaching of foliar nutrients is a normal part of nutrient cycling, increased acid deposition may accelerate normal leaching rates to the point where critical internal nutrient balance is jeopardized (Carey et al. 1986). One of the results of leaching of  $Mg^{2+}$  can be a concurrent reduction in chlorophyll (Malhotra 1976, Jaakkola et al. 1980). Reductions in chlorophyll can result in decreased photosynthesis and, ultimately, in decreased growth and biomass production (Houpis et al. 1988). The extent to which acid precipitation results in increased foliar leaching varies as several western conifer species showed no leaching response while some short term nutrient reductions were observed for red spruce (Peterson et al. 1991).

Several researchers have demonstrated that dry acid deposition adversely affects stomatal physiology (Caput et al. 1978, Beckerson and Hofstra 1979, Bytnerowicz and Taylor 1983), but the effect of wet deposition on stomatal conductance and transpiration is less certain. One study indicated that acid precipitation with pH values as low as 3.5 had no effect on the transpiration of several tree species (Kelly et al. 1984). However, indirect evidence has shown that acid precipitation may affect stomatal function by directly affecting the guard cells (Dybing and Currier 1961, Evans et al. 1977, 1978; Evans and Curry 1979). Effects on stomata may secondarily affect photosynthesis as well. If conductance increases, the internal water status of the plant may be altered and an increased flux of other pollutants, such as  $O_3$ , may occur. As a result, photosynthesis may be reduced. Some studies have shown negligible direct acid precipitation effects on photosynthesis (Reich and Amundson 1985, Reich et al. 1986).

Reports of the effects of acid precipitation on growth have been variable. Lee and Weber (1979), working with 11 North American tree and shrub species, found no reduction in growth at pH levels down to 3.0 for a majority of the species. Tingey and Hogsett (as reported by Peterson et al. 1991) observed increased height and diameter growth of *P. ponderosa* seedlings at pH 3.1 compared to pH 5.6. Percy (1982) did not find growth reductions in spruce (*Picea glauca* and *Picea rubens*) at pH levels down to 3.0, but did report significant reductions at pH levels less than 3.0. Differences in the N and S balance of applied solutions may be partly responsible for the observed variation in results.

#### B. Ozone

Ozone is known to adversely affect the vigor and productivity of many forest species through directly impacting several physiological and biochemical processes. These include decreases in photosynthesis and possible alterations of stomatal conductance (Hallgren et al. 1982, Mann et al. 1980, Noyes 1980), increased respiration (Barnes 1972, Edwards et al. 1992), alteration of carbon allocation patterns (Mahoney et al. 1985, Friend and Tomlinson 1992, Friend et al. 1992), reductions in foliar pigmentation (Sasek et al. 1991), stimulation of foliar abscission (Gunthardt-Goerg et al. 1993), reduction in foliar retention (Miller et al. 1983), increases in membrane permeability, and a decrease in photosynthetic pigmentation (Heath 1980).

Several studies demonstrate physiological evidence for growth reduction due to ozone (Tingey *et al.* 1976, Skeffington and Roberts 1985). Similar trends in the effects of ozone on photosynthesis and some parameter of dry weight accumulation are common (Barnes 1972, McLaughlin *et al.* 1982, Reich and Amundson 1985). It has been demonstrated that exposure to elevated ozone concentrations may result in reduced translocation of assimilated carbon from leaves to stems and roots (Edwards *et al.* 1992) as a greater proportion of carbon may be partitioned into organic acids, lipids, pigments and residues (Friend and Tomlinson 1992). Such changes in carbon allocation patterns have lead to a reduced proportion of dry weight in the roots (Hogsett *et al.* 1985, Mahoney *et al.* 1985). Yang *et al.* (1983) concluded that ozone-induced alteration in biomass production is a function of a number of parameters, including photosynthesis, chlorophyll content, membrane permeability, translocation, and leaf area.

Several investigations have indicated that gas-exchange processes may be influenced by ozone exposure. Reductions in photosynthetic rates and capacities have been observed following prolonged exposure to relatively mild ozone concentrations for a number of forest species including loblolly pine (Sasek and Richardson 1989, Sasek *et al.* 1991), ponderosa pine (Miller *et al.* 1969, Coyne and Bingham 1981, Beyers *et al.* 1992), Fraser fir (Tseng *et al.* 1988) and eastern white pine (Reich and Amundson 1985). Although, a lack of photosynthesis response to ozone has been observed in some studies including one using ponderosa pine seedlings (Bytnerowicz *et al.* 1989). Reductions in stomatal conductance have also been shown to accompany ozone exposure (Miller *et al.* 1978, Coyne and Bingham, 1982).

### C. Acid Rain and Ozone Combined Effects

It is also possible that direct acid precipitation effects can be more severe if coupled with exposure of the foliage to O<sub>3</sub> (Hileman 1984, Hart *et al.* 1986). One pollutant may predispose foliage to increased damage by another, or two pollutants together can affect physiological processes to a greater degree than that possible from either pollutant alone. Ozone may increase cell membrane permeability resulting in increased acid precipitation-induced leaching of ions (particularly Mg<sup>2+</sup> and Ca<sup>2+</sup>). The leaching can result in nutrient deficiency, reductions in photosynthesis and root production, and to reduced growth and nutrient uptake (Hileman 1984, Skeffington and Roberts 1985, Hart *et al.* 1986). Interestingly, several seedling studies have shown slight increases in plant growth under limited conditions of either ozone or acid rain exposure suggesting that both pollutants may provide a fertilization effect under specific circumstances (Peterson *et al.* 1991).

### D. Gas Exchange and the Impact of Atmospheric Pollution on Forest Vegetation

Gas-exchange characteristics are important in determining the potential impact of pollutant exposure on forest vegetation. The basic mechanisms for carbon fixation by leaves require the exchange of CO<sub>2</sub> from the atmosphere to the sub-stomatal cavity, and

eventually to the mesophyll of leaves. Stomatal aperture and regulation are required to provide a pathway for CO<sub>2</sub> exchange. Ideally, a plant that has relatively high stomatal conductance will have greater potential for CO<sub>2</sub> uptake. Yet, this pathway is shared by other atmospheric constituents including oxidizing agents such as ozone. It has long been noted that there often exists strong correlations between trees with high stomatal conductance rates and the degree of foliar oxidant damage and relative reduction in photosynthesis (Miller *et al.* 1978, Coyne and Bingham 1982). Stomatal sensitivity to environmental and pollutant conditions may ultimately determine the internal pollutant dosage experienced by foliage. These observations have provided researchers a basic working hypothesis for the past 15 years and in many situations it appears to have been validated.

Several questions persist regarding variability in plant response to air pollutants and the role of gas-exchange processes. Much of the work to date has been based on seedlings. Given that ozone, and to a lesser degree, acid rain influence carbon allocation, it is important to examine the responses of mature trees to these pollutants as the relative sizes of various carbon pools, and therefore, carbon source-sink relationships, can be expected to differ markedly between seedling and mature life-stages. Translocation rates of carbon compounds and growth regulators from sources to sinks may differ on the order of days between seedlings and mature trees simply because of the difference in canopy to root path-length (Cregg *et al.* 1989). Gas-exchange processes may also differ among life-stages as previous work with loblolly pine (Cregg *et al.* 1989) and with ponderosa pine (Anderson *et al.* 1990) have demonstrated that seedling foliage maintained generally higher photosynthesis and conductance rates than mature tree foliage.

Gas exchange responses to ozone exposure have been shown to vary among genotypes within species (Coyne and Bingham 1982, Sasek *et al.* 1991). As with any stress, variability in response suggests that plant mechanisms of tolerance or avoidance may be operating to different degrees among genotypes. Earlier efforts observed that photosynthetic rates and phenotypic vigor were negatively correlated with extent of foliar ozone damage and needle retention for mature ponderosa pine of three clones (Anderson *et al.* 1990). Friend and Tomlinson (1992) found that under ozone stress, carbohydrates were partitioned to a greater degree to lipids, pigments, organic acids and residues; an indication of repair response. It may be hypothesized that some genotypes exhibit stress tolerance responses more strongly than stress avoidance responses. Maintenance of high photosynthesis and conductance rates would favor stress tolerance responses by maintaining a supply of carbon for the production of repair compounds.

Many studies examining photosynthesis and stomatal conductance response to atmospheric pollutants have conclusions based on measurements made under a predefined set of optimal conditions. Such studies use maximum gas exchange rates as the point of reference for determining pollutant effect. To assess potential pollution effects on forest vegetation, it is necessary to consider the range of environmental conditions to which plants will respond since, for much of the time, plants are exposed to suboptimal microclimatic conditions. The question arises as to how variation in environmental conditions will influence potential pollutant uptake. Conversely, given

that plants have been exposed to a given pollutant regime, we need to consider how gas-exchange responses vary with momentary, diurnal and seasonal variations in microclimate.

## II. Research Objectives

Our objectives in performing this research were to:

- 1) Identify photosynthesis, stomatal conductance and foliar pigmentation responses of *Pinus ponderosa* foliage to long-term realistic ozone and acid rain exposure.
- 2) Characterize seasonal variation in gas-exchange response to pollutant exposure.
- 3) Evaluate the relative physiological and growth responses by seedlings and mature tree branches to acid rain and ozone.
- 4) Identify within species variation in the gas-exchange response of *Pinus ponderosa* to pollutant exposure.
- 5) Develop gas exchange environmental response surface models that may potentially be used in the development of a process model of pollutant uptake by forest vegetation.

## III. Materials and Methods

### A. Chico Air Pollution and Climate Change Facility

The study was performed at the Chico Air Pollution and Climate Change Research Facility (CAPACC) located at the U.S. Forest Service Tree Improvement Center (CTIC) in Chico, CA. The facility is an established laboratory for conducting large-scale outdoor exposure studies and is uniquely suited to addressing the issue of life-stage comparability due to the availability of both seedlings and mature trees of known genetic origin. Mature trees of graft origin and corresponding one-half sibling seedlings are available for experimentation. The principal benefit of having experimental material of known genetic origin is decreased among-family variability as a source of experimental error. This provides increased precision of parameter estimates and, consequently, effects of the air pollution treatments may be discerned statistically with fewer replications. Comparisons of mature branch and seedling foliage response are more robust as individuals representing both life stages are derived from a common parent.

## B. Plant Materials

### 1. Mature tree clones

Branches of trees of graft origin at the CTIC were used as the source of mature tissue in this study. Buds from forest-grown, 70- to 80-year-old ponderosa pine, identified by the United States Forest Service (USFS) as having superior growth and form characteristics, were grafted onto 3-year-old root stocks. The grafts were out-planted (4.25 m x 4.25 m spacing) to their current location in the CTIC seed production orchard between 1977 and 1978 (Figure 1). These trees are currently 7.5 to 12 m in height and as large as 30 cm in diameter. The grafted trees retain morphological and physiological characteristics of 80-year-old trees. This is evidenced by characteristics of branch diameter, needle length, and sexual maturity. Graft rejection in *P. ponderosa* is generally evident within five years of propagation, thus the study trees are viable individuals.

Six mature trees (ramets) each of three genotypes (18 trees total) were fitted with BECs and necessary fumigation and monitoring equipment for air pollution treatment application. The three genotypes (clones 3087, 3088, and 3399) originated from three parent trees located at 1190 m to 1220 m elevation on the Eldorado National Forest in the central Sierra Nevada (USFS seed zone 526). Relative to other genotypes in the production orchard at CTIC originating from the same elevation and breeding zone, these families represent the full range of phenotypic vigor as defined by overall size, branch development, foliage density, foliage retention, and foliage color. Trees of clone 3088 have the least vigor and trees of clone 3399 have the greatest vigor.

Previous work has shown that these three families differ physiologically in terms of gas-exchange rates, pigmentation and nutrient status (Anderson *et al.* 1990). Morphologically, clone 3088 is of smaller stature and retains one to two age-classes of foliage. Clone 3399, which has higher gas exchange rates, has greater foliar concentrations of chlorophyll a, carotenoids and nitrogen, tends to have greater height and crown expanse, and typically retains two to three age-classes of foliage.

### 2. Half-sib seedlings

Half-sib seedlings from clones 3087, 3088 and 3399 were used as the source of seedling tissue. Open-pollinated cones were collected from the same superior trees from which buds were collected for grafting. Seedlings produced from this seed are directly related (half-sibs) to the mature tree clones. The 10-month-old container-grown seedlings were planted in an area adjacent to the mature tree production orchard in February, 1990. One seedling of each genotype was planted at 36 branch exposure chamber (BEC) locations and at six non-chambered companion plot locations. The seedling BECs and companion plots have a spacing of 4.6 m between rows and 2 m between BECs within rows (Figure 1). The three seedlings per BEC or companion plot were planted in a triangular pattern at a 30 cm spacing. Planting one seedling per genotype per BEC or companion plot, was done to provide four replications of the nine

possible acid rain x ozone treatment combinations for each genotype. Given that 36 BECs were available for exposing seedlings, less than two replications were possible if each BEC was used to expose one seedling of one genotype.

Although the growing space within the chamber was fully occupied by the end of the 1992 growing season, irrigation and fertilization were used to mitigate among-tree competition for moisture and nutrients. Through the 1991 growing season, the small stature of the seedlings resulted in very little interplant shading. By the middle of the 1992 growing season, some interplant shading did occur but this was minimized by the orientation of the triangular planting arrangement. During mid-day gas-exchange measurements, all of the seedlings received full illumination over most of the crown. During the 1992 growing season, within-tree shading of one-year-old foliage was more problematic for sample selection than was among-tree shading.

### 3. Cultural practices

Because the mature trees used in this study are part of an operational seed production orchard, cultural activities (irrigation, pest control, fertilization) applied to the experimental trees were the same as those for the entire orchard. The trees were irrigated from May through October to minimize moisture stress. The irrigation regimen consisted of the application of 5 to 7 cm water at 10 to 14 day intervals for the mature trees and weekly for seedlings. Fertilization occurred once in the spring of 1992 with nitrogen ( $148 \text{ kg ha}^{-1}$ ), phosphorus ( $25 \text{ kg ha}^{-1}$ ), sulfur ( $204 \text{ kg ha}^{-1}$ ), calcium ( $7.6 \text{ kg ha}^{-1}$ ), manganese ( $15.8 \text{ kg ha}^{-1}$ ) and magnesium ( $0.1 \text{ kg ha}^{-1}$ ). Herbaceous ground cover in a 1.5 m wide strip within planted rows was eliminated with glyphosate herbicide.

#### C. Air pollution exposures

Ozone and acid rain treatments were applied individually and in combination to both mature branches and seedlings. The timing and levels of exposure were designed to mimic, to the extent logistically possible, periodicities of pollutant exposure occurring in the natural forest environment of the central Sierra Nevada. Ozone was applied year-round with treatment levels defined as multiples of the ambient concentration that tracked diurnal and seasonal variation. Simulated acid rain treatments were applied during the winter to coincide with the natural precipitation season. For both ozone and acid rain exposures, the most extreme treatment levels applied represent chronic exposures that have reasonably high probability for occurring at present or in the near future given demographic trends for central and northern California.

#### 1. Acid Rain Treatments

Acid rain applications were carried out for a four month rain season from mid-January through mid-May, 1992. The rain treatment levels consisted of: 1) simulated rain of pH 5.1; 2) simulated rain of pH 3.0; and 3) no acid rain (natural rain was excluded by the BEC).

The rainfall solution used was the same as that described by McColl and Johnson (1993). Trace quantities of salts were added to deionized water in fixed proportions to mimic rainfall chemistry as measured in the Sierra Nevada (McColl and Johnson 1983). The pH 5.1 solution was prepared by aerating the salt solution and allowing it to incorporate CO<sub>2</sub> from the atmosphere and form carbonic acid. The pH 3.0 solution was prepared by adding H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> in a fixed ratio (2:3 equivalent basis, 1:3 volumetric basis) to the salt solution, again mimicking mean SO<sub>4</sub><sup>2-</sup> and NO<sub>3</sub><sup>-</sup> ratios as determined from Sierra Nevada rain samples (McColl and Johnson 1983). The nominal concentrations and deposition quantities for ions in the acid rain solutions are presented in Table 1. Budgetary constraints prohibited composition analyses of the solutions used in this study. Analyses of solutions produced during a 1990 study using the same protocol confirm the chemical composition of the solutions (Houpis and Anderson, unpublished data). Details of the solution preparation protocol are presented as Appendix A.

The rain season consisted of 17 rain events, occurring at an average interval of 5.6 d, in which 5 cm of precipitation was applied per event (Table 2). A total of 85 cm of rain was applied. The applied solution was collected as it drained from the bottom of the BEC and disposed of away from the trees or seedlings. This was done to prevent the rain solution from modifying the soil chemistry and possibly confounding foliar morphological and physiological responses to the various treatment combinations. No attempt was made to measure foliar interception, throughfall deposition or alterations of solution chemistry as these parameters, although potentially informative, were beyond the scope and funding levels of the study. The efficiency of the catchment systems (solution collection) averaged 85 percent for the trees and 81 percent for the seedlings (Table 2).

The rain solution was applied through a stainless steel nozzle (model WL-1/4-80, Bete Fog, Inc., Greenfield, MA) mounted in the center of the BEC 15 cm below the baffle plate. The rain solution was supplied at a line pressure of 10 psi which resulted in a mean flow rate of  $0.626 \pm 0.076$  l min<sup>-1</sup>. The mean droplet size of the spray was 302  $\mu$ m with 80 percent of the droplets having diameters between 207 and 579  $\mu$ m (data from Bete Fog, Inc.). The mean droplet size for naturally occurring rain has a range of 100 to 1000  $\mu$ m (Hart *et al.* 1986, Reich *et al.* 1986). Previous laboratory testing of the spray distribution pattern within the BEC indicated that spray deposition rate at the base of the BEC increased asymptotically over the 35 cm radial distance; from 8 to 18 mm h<sup>-1</sup> for the 0 to 10 cm radial distance and from 18 to 21 mm h<sup>-1</sup> for the 10 to 30 cm radial distance (Anderson, P.D., unpublished data).

The 5 cm deposition per event was calculated relative to the plane 60 cm above the base of the BEC which corresponded to the approximate height of the seedling terminal shoot in January, 1992. To achieve 5 cm deposition, a total of 22.4 l of solution were delivered to each BEC. Given that the basal area of a BEC is 0.385 m<sup>2</sup> and a deposition of 5 cm, the minimum volume of solution required per event, under conditions of strictly vertical deposition, was 19.2 l. Since the spray angle of the nozzles was 80 degrees, some of the solution was sprayed onto the sides of the chamber and thus the actual volume required to achieve the vertical deposition of 5 cm exceeded the minimum volume by 3.2 l. The mean delivery rate to the BECs was 5.8 cm h<sup>-1</sup>.

## 2. Ozone Fumigation

Mature branches and seedlings were exposed to three ozone treatment levels in the study, including: 1) charcoal-filtered ambient (CF), ambient (AMB) and twice ambient (2xAMB). In addition, non-chambered companion branches and companion seedlings were included for assessing effects due to the BEC.

The CF exposure consisted of passing ambient air through an activated charcoal filter in addition to the particulate filter contained on all BEC fan-boxes. The charcoal filters reduced the ozone concentration in the BECs to 40 to 60 percent of the ozone concentration of ambient air. The CF treatment provides a basis for assessing potential responses to a cleaner ozone environment.

The 2xAMB level was a multiplicative value of the ambient ozone concentration. A multiplicative exposure factor was chosen over an additive exposure factor for two reasons: First, the use of a multiplicative factor allowed applied ozone concentrations to follow diurnal trends of ozone concentration found in the ambient environment. Second, the use of a multiplicative factor prevented the occurrence of abnormally high applied ozone concentrations in the morning and evening hours that would have occurred had an additive factor been used.

The CF and AMB treatments were passive fumigations, requiring only periodic maintenance of particle and charcoal filters. The 2 x AMB treatment was applied by generating ozone from compressed air using an O<sub>3</sub> generator (Model GL-1, PCI, Inc., New Jersey). Ozone enrichment of the fumigation air stream was automatically controlled by computer based on continuous monitoring of ambient ozone concentrations (Houpis *et al.* 1988). Exposures were conducted from 0600 to 2000 h daily throughout the study period. This exposure regimen allowed the tracking of both diurnal and seasonal variation in ambient O<sub>3</sub> concentration.

### D. Atmospheric Monitoring and Data Acquisition

Sampling of chamber atmospheres was conducted through 5 µm Teflon particle filters and Teflon tubing that was calibrated for O<sub>3</sub> loss. Air samples were drawn continuously from each BEC at a rate of 3 l min<sup>-1</sup> to 12-position sampling valves (Scanivalve, Inc., San Diego, CA) and continuously exhausted unless selected for analysis. Each chamber atmosphere was analyzed for 1.25 minutes four times per hour.

The 90 BECs and eleven ambient sample lines required nine sampling valves for monitoring. Nine ozone analyzers (one per sampling valve) were employed. Large-volume sampling pumps were used to pull chamber and ambient samples to analyzers housed in climate-controlled instrument shelters. Once selected for analysis, samples were drawn to the O<sub>3</sub> monitors by internal pumps. The above sampling scheme allowed for a minimum of one-hour averaging of O<sub>3</sub> concentrations.

Ozone monitoring took place in two small buildings and one trailer spaced throughout the field site (Figure 1). This was done so that all sample lines were less than 80 m in length from BEC to analyzer to avoid excessive line-loss of O<sub>3</sub>. All data acquisition took place in the monitoring trailer. The two buildings and monitoring trailer

were temperature-controlled. Ozone fumigations were conducted from a separate trailer, to avoid contamination of monitoring systems.

A computer-controlled data acquisition system (DAS; Model 3497A control unit and Model 3498 extender unit, Hewlett-Packard, Inc., Loveland, CO) was used to control the sequencing of sampling-valves and collection of the chamber and ambient ozone data. Error-checking of sampling-valve positions during each monitoring step ensured that measurements were conducted on the correct chamber and ambient samples.

Air temperature (for each BEC and the ambient environment) and soil temperature were also monitored using type-T (constantan-chromel) thermocouple sensors interfaced with the DAS.

Analysis of chamber and ambient ozone samples was accomplished using nine ozone analyzers. Eleven ozone analyzers (two as spares) were available for sampling and an additional ozone analyzer was dedicated to use as a calibration transfer standard. Eight of the monitoring analyzers were Manufactured by Dasibi, Inc. (Model 1003). Two monitoring analyzers and the transfer standard analyzer were manufactured by Environics, Inc. (Series 300).

## E. Physiological Measurements

In the proposed study, we evaluated gas exchange characteristics in two ways: 1) we monitored monthly mid-day values of net photosynthesis and stomatal conductance; and 2) we developed net photosynthesis and stomatal conductance environmental response surfaces. Monthly measurements of mid-day net photosynthesis and stomatal conductance provide a means for monitoring physiological responses to seasonal variation in environment that include the effects of phenological state and tissue maturation. The environmental response surfaces provide functional relationships among net photosynthesis or stomatal conductance and environmental conditions of light intensity and temperature. The functional relationships provide a means for predicting gas exchange rates over a variety of light and temperature conditions.

### 1. Mid-day Gas-exchange Methodology

Mid-day net-photosynthesis ( $P_n$ ) and stomatal conductance ( $g_s$ ) were measured on a monthly basis using a portable closed-loop gas exchange system (LI-6200, Licor, Inc., Lincoln, NB). Measurements were made under ambient temperature and humidity conditions on fully exposed foliage. One fascicle was enclosed in the cuvette. Instantaneous estimates of  $P_n$  and  $g_s$  were made over a 20 to 30 s period where cuvette relative humidity was maintained within 2 percent of ambient and the cuvette  $CO_2$  draw-down was in the range of 4 to 10 ppm.

Two observations each were made on current-year (February through October) and one-year-old foliage (June through November) for each experimental branch or seedling. For each foliage age-class, one replication of mature branches and two replications of seedlings were measured during each of two days per month. Due to logistical constraints, it was not possible to sample foliage of both age-classes on the

same day. Thus, one foliage age-class was measured per day and a total of four days per month (June through October) were required to complete the mid-day measurements. The 216 measurements per day were made during a 2.5 hour interval beginning at 1100 h PST or 1000 h PDT using three photosynthesis systems. Foliar surface area of the sample tissue was determined non-destructively using established optical methods (Bingham as presented in Licor 1983). These methods involved the measurement of needle length and chord, and calculation of surface area according to the formula:

$$FSA = L \cdot (1 + 2 \cdot [\pi / N]) \cdot h / \sin(180 / N)$$

where:

FSA = the total surface area of all needles in the fascicle (cm<sup>2</sup>).

L = the length of needles enclosed in the cuvette (cm)

N = the number of needles per fascicle.

h = the chord of the needles (cm).

Three LI-6200 systems were used concurrently to collect the gas-exchange data. All system sensors (thermocouple, humidity sensor, mass flow meter, quantum sensor) were factory calibrated in December, 1991, prior to initiation of data collection. Each LI-6250 infra-red gas analyzer (IRGA) was calibrated at the beginning of each daily measurement period against a calibration gas of known concentration traceable to the National Bureau of Standards. Thermocouple and humidity sensor calibration were checked prior to mid-day measurements in March and August of 1992 and adjusted as necessary. Thermocouples from the three systems were concurrently calibrated against a digital thermometer. The humidity sensors were calibrated using a series of air streams of known humidities produced by a dewpoint generator (Model LI-610, Licor Inc., Lincoln, NB).

For each measurement period, each treatment level and life-stage was sampled equally among the three LI-6200 systems to avoid instrument bias of the observations. As a result, variation among observations due to differences in sensor performance were distributed among all experimental factors and, therefore, were treated as experimental error. Standardization of instrument performance based on measurement of common foliage samples was not performed. Repeated measures of foliage over a short time span result in alteration of foliage gas exchange rates and over longer time spans, microclimate and tissue process rates fluctuate.

## 2. Response Surface Gas-exchange Methodology

Environmental response surface development consisted of measuring CO<sub>2</sub> and water vapor flux on tissues acclimated to controlled temperature, light and vapor pressure deficit conditions. These measurements, made in late summer (August 18-

September 7) and repeated in the fall (November 2-14), provide data for the construction of environmental response surfaces. Net photosynthesis and  $g_s$  were determined at all combinations of three light intensity levels (250, 500 and 1000  $\mu\text{E m}^{-2} \text{s}^{-1}$ ) and three cuvette temperatures (18, 25 and 32 °C). Response surfaces were developed for all combinations of AMB ozone, 2xAMB ozone, pH 5.1 acid rain, and pH 3.0 acid rain. In the late summer period, measurements were made for both current-year and one-year-old tissue. In the fall period, measurements were made for current-year foliage only as the one-year-old foliage had senesced on several seedlings of all clones and on mature branches of clone 3088.

Sample tissue consisting of 2 to 3 fascicles were placed in the 0.86 l cuvette and measurements for all nine combinations of light and temperature were made. The measurements were made in a sequence consisting of progressive increases in light intensity within each level of temperature. Thus, once the cuvette temperature was stabilized at one of the three levels, measurements were made at light levels of 250, 500 and 1000  $\mu\text{E m}^{-2} \text{s}^{-1}$ ; with each measurement preceded by a 10 to 15 min acclimation period. A 30 to 45 minute stabilization period was used between cuvette temperature changes. Vapor pressure deficit in the chamber was adjusted to maintain a target value of 1.0 kPa during the acclimation period.

Response surface measurements were made using a system consisting of a temperature controlled cuvette (DDG-9920, Data Design Group, La Jolla, CA) and an infra-red gas analyzer (Li-6250, Licor, Inc., Lincoln, NB). Temperature of the cuvette was maintained at a set-point value using a temperature controller (CN9111, Omega Engineering, Inc., Stamford, CT). Vapor pressure deficit within the chamber during acclimation was maintained constant using a dew-point generator (Licor Inc., Lincoln, NB). Photosynthetically active light intensity within the cuvette was controlled using an external supplemental light source consisting of two 27 Watt fluorescent lamps arrayed parallel to the top surface of the cuvette. The light levels were varied by changing the height of the light source above the cuvette.

### 3. Pigmentation Analysis

Foliar samples were analyzed for chlorophyll a, chlorophyll b, and carotenoid content. Sampling was performed during May and September in conjunction with monthly mid-day gas exchange measurements. An N,N-dimethyl formamide (DMF) extraction method was used, which enabled us to easily process and analyze 100 samples per day (Moran and Porath, 1980). One fascicle per seedling or branch was collected, measured for leaf area, cut into 1 cm segments and immersed in 5 ml DMF extraction solvent. The samples were kept in the dark at 4 °C during a 21 d pigment extraction period. Following extraction, the absorbance of the extract was measured spectrophotometrically at wavelengths of 440 nm, 645 nm and 662 nm using a diode array spectrometer (Model 8452a, Hewlett-Packard, Inc.). The total content of chlorophylls a and b and carotenoids was calculated based on absorbance coefficients of Lichtenthaler and Wellburn (1983). Pigment concentrations were expressed on a leaf area basis.

## F. Morphological Measurements

Repeated measurements of diameter and stem length were made for both mature branches and seedlings over the study period. Diameter was measured at the cotyledon scar for seedlings and at the base of the 1991 stem segment for the mature branches. Diameter measurements were made at approximate monthly intervals beginning in August 1991. Measurements of seedling terminal shoot length and mature branch length were made from February through November, 1992. From February through August, length measurements were made at approximate two-week intervals and then monthly thereafter. For both seedlings and branches, stem length was measured from the base of the 1992 growth to the tip of the terminal bud on the terminal shoot (or a dominant lateral shoot if the terminal shoot was missing or damaged).

## G. Statistical Design and Analyses

### 1. General Models

The study evaluates the effects of three factors, ozone (3 levels), acid rain (3 levels) and genotype (3 levels), on gas-exchange and growth characteristics for foliage of two age-classes of two life-stages of *Pinus ponderosa*. For both mature branches and seedlings, the treatments were allocated in a split-plot factorial design. Due to differences in the treatment structure between the seedling and mature branch life-stages, two statistical models must be used in the analyses to correctly reflect differences in factor nesting. As a result, statistical comparisons of seedling and mature branch responses to the effects of ozone exposure and genotype are precluded.

For mature branches, the largest experimental unit (whole-plot) was the tree. Each tree represented one of three levels of genotype. The three levels of acid rain were assigned at random to each tree or whole-plot. Three branches within a tree represented sub-plots to which the three levels of ozone were assigned at random; each level of ozone was assigned to one branch per tree. The ozone treatment was a split-plot factor within a genotype x acid rain complete factorial treatment structure applied to the tree whole-plots. There were six ramets (clonal trees) of each of three genotypes for a total of 18 whole-plots. Thus, each of 54 branch sub-plots received one of nine possible combinations of ozone and acid rain treatment. In addition, each of the 18 trees had one non-chambered, untreated branch that served as a control. Therefore, for mature trees, the experiment consisted of a total of 72 branch sub-plot experimental units providing two replications of 36 acid rain x genotype x ozone treatment combinations.

The whole-plot experimental unit for seedlings was the BEC (exposure chamber). Acid rain and ozone treatments were assigned as a completely randomized 3 x 3 factorial to the seedling BECs. Within each BEC, three half-sib seedlings, one of each of three genotypes, constituted split-plot experimental units. There was a total of 36 seedling BECs providing four whole-plot replications of the 9 ozone and acid rain treatment combinations. In addition, there were six non-chambered control groups of three seedlings.

Valid analyses of the data required the use of different models for the two life-stages to account for the differences in treatment structure. For mature branches, the general model used (excluding interaction terms for clarity) was:

$$Y_{ijkl} = \mu + A_i + G_j + e_{ijk} + O_l + \delta_{ijkl}$$

where:

- $Y_{ijkl}$  = is the observed parameter value for the  $l$ th ozone level, of the  $k$ th replication, of the  $j$ th genotype, of the  $i$ th acid rain treatment
- $\mu$  = the population mean for the parameter
- $A_i$  = the effect of  $i$ th acid rain treatment
- $G_j$  = the effect of the  $j$ th genotype
- $e_{ijk}$  = the whole-plot error
- $O_l$  = the effect of the  $l$ th ozone treatment
- $\delta_{ijkl}$  = the sub-plot error

For seedlings, the general model used (excluding interaction terms) was:

$$Y_{ijkl} = \mu + A_i + O_j + e_{ijk} + G_l + \delta_{ijkl}$$

where:

- $Y_{ijkl}$  = is the observed parameter value for the  $l$ th ozone level, of the  $k$ th replication, of the  $j$ th genotype, of the  $i$ th acid rain treatment
- $\mu$  = the population mean for the parameter
- $A_i$  = the effect of  $i$ th acid rain treatment
- $O_j$  = the effect of the  $j$ th ozone treatment
- $e_{ijk}$  = the whole-plot error
- $G_l$  = the effect of the  $l$ th genotype
- $\delta_{ijkl}$  = the sub-plot error

The general structure of the ANOVA applied to the mature branch and seedling models is presented in Table 3.

## 2. Mid-day Gas Exchange Analysis

For mid-day gas exchange analyses, inclusion of foliage age-class as a factor in the model is not valid as current-year and one-year-old foliage were measured on different days. Thus, inferences concerning the effects of foliage-age on mid-day gas exchange may be made based on subjective evaluation of the data with the understanding that

comparability depends on the degree of environmental uniformity between measurement dates and that a statistical probability of significance cannot be assigned to observed differences.

To account statistically for variation over the entire study period, monthly mid-day gas exchange data were subjected to repeated measures ANOVA (RMANOVA). A univariate approach was employed. The models employed were derivations from the general models for mature branches and seedlings. The univariate RMANOVA model (excluding interaction terms) applied to the analysis of mature branch data is presented below:

$$Y_{ijklm} = \mu + A_i + G_j + e_{ijk} + O_l + \gamma_{ijkl} + T_m + \lambda_{ijkm} + \delta_{ijklm}$$

where:

- $Y_{ijklm}$  = is the observed parameter value for the mth measurement period, of the lth ozone level, of the kth replication, of the jth genotype, of the ith acid rain treatment.
- $\mu$  = the population mean for the parameter
- $A_i$  = the effect of ith acid rain treatment
- $G_j$  = the effect of the jth genotype
- $e_{ijk}$  = the whole-plot error
- $O_l$  = the effect of the lth ozone treatment
- $\delta_{ijkl}$  = the ozone sub-plot error
- $T_m$  = the effect of the mth measurement period
- $\lambda_{ijkm}$  = the measurement period sub-plot error
- $\delta_{ijklm}$  = the sub-plot error

The model for seedling RMANOVA was very similar with the difference being a reversal of the nesting of the ozone and genotype effects. The general structure of the RMANOVA applied to the mature branch and seedling models is presented in Table 4.

### 3. Environmental Response Surface Gas Exchange Analysis

Analyses of the stomatal conductance and photosynthesis environmental response surface data were accomplished in two steps. First, analysis of covariance (ANACOV) was used to identify significant sources of variation in the data. Secondly, response surfaces were fit to the data grouped by significant main and interaction classification effects.

The ANACOV models used to evaluate sources of variation in the response surface data differed from the models used in the analysis of mid-day gas exchange in that there was no repeated measures term and variables representing the effects of environment (light intensity, cuvette temperature and vapor pressure deficit) were included as covariates. Although the study was designed to hold vapor pressure deficit

(VPD) constant for all combinations of light and cuvette temperature, examination of the response surface data revealed that the variation in VPD about the target value was a significant source of variation in the observed values of stomatal conductance and, to a lesser extent, photosynthesis. Thus, VPD was included as a covariate in the ANACOV model. The covariates were entered at the second-order level. The ANACOV model (excluding interaction terms) used for evaluating seedling data is presented below:

$$Y_{ijkl} = \mu + A_i + O_j + \epsilon_{ijk} + G_l + \beta_{ijkl} + \eta + \eta^2 + \kappa + \kappa^2 + \omega + \omega^2 + \eta\kappa + \eta\omega + \kappa\omega$$

where:

- $Y_{ijkl}$  = is the observed parameter value for the  $l$ th genotype, of the  $k$ th replication, of the  $j$ th ozone level, of the  $i$ th acid rain treatment
- $\mu$  = the population mean for the parameter
- $A_i$  = the effect of  $i$ th acid rain treatment
- $O_j$  = the effect of the  $j$ th ozone treatment
- $\epsilon_{ijk}$  = the whole-plot error
- $B_l$  = the effect of the  $l$ th genotype
- $b_{ijkl}$  = the genotype sub-plot error
- $\eta$  = the effect of light intensity covariate
- $\kappa$  = the effect of cuvette temperature covariate
- $\omega$  = the effect of vapor pressure deficit covariate

The model for mature branch ANACOV was very similar with the difference being a reversal of the nesting of the genotype and ozone effects.

For selected significant acid rain, ozone, genotype, and foliage age-class main effects and interactions, environmental response surface models were fit using a second-order polynomial regression equation with light intensity, cuvette temperature and vapor pressure deficit as the independent variables. To determine differences among fitted equations for each significant effect, Bonferroni confidence intervals with an experiment-wise error rate of  $p=0.05$  were calculated for each parameter in the models (Neter and Wasserman 1974). Differences in parameter estimates among the fitted models were identified by non-overlapping Bonferroni confidence intervals.

#### 4. Seedling and Mature Branch Growth Analysis

Diameter growth and stem elongation of seedlings and mature branches were analyzed using RMANOVA procedures. The statistical model used was essentially the same as that applied to the mid-day gas exchange data (see Table 4) with the difference being the number of measurement periods. Separate analyses were performed for the mature branch and seedling lifestages due to constraints of experimental design discussed previously.

Absolute measures of diameter and stem length were normalized relative to values for the first measurement event. This was done to account for the effect of variation in initial size on subsequent growth. The dependent variables for diameter and height, therefore, were cumulative percent increase in dimension with respect to 1) basal diameter of seedlings; 2) basal diameter of the 1991 branch stem segment; 3) seedling total height prior to 1992 bud-break; or 4) the length of the 1991 branch segment. As defined, the normalized variables for seedlings represent percent growth relative to total size prior to the study while for mature branches, normalized variables represent percent growth relative to dimensions of tissue produced during 1991.

Table 1. Nominal ionic composition and deposition of acid rain solutions.

Acid Rain Solution Ionic Composition and Deposition							
Ion	Source	Soln. Conc. ( $\mu\text{eq l}^{-1}$ )		Deposition per Event (meq)		Total Deposition (meq)	
		pH 5.1	pH 3.0	pH 5.1	pH 3.0	pH 5.1	pH 3.0
$\text{H}^+$	$\text{H}_2\text{SO}_4$ & $\text{HNO}_3$	7.6	1000	0.15	19.2	2.55	326.4
$\text{Mg}^{+2}$	$\text{Mg}(\text{NO}_3)$ $6\text{H}_2\text{O}$	6.0	6.0	0.12	0.12	2.04	2.04
$\text{Ca}^{+2}$	$\text{CaCl}_2$ $2\text{H}_2\text{O}$	14.8	14.8	0.28	0.28	4.76	4.76
$\text{NH}_4^+$	$(\text{NH}_4)\text{SO}_4$	28.0	28.0	0.54	0.54	9.18	9.18
$\text{K}^+$	$\text{K}_2\text{SO}_4$	1.5	1.5	0.03	0.03	0.51	0.51
$\text{Cl}^-$	$\text{CaCl}_2$ $2\text{H}_2\text{O}$	14.8	14.8	0.28	0.28	4.76	4.76
$\text{HSO}_4^-$	$\text{H}_2\text{SO}_4$	0.0	166	0.00	3.19	0.00	0.00
$\text{SO}_4^-$	$\text{H}_2\text{SO}_4$	29.5	199	0.57	3.82	9.69	9.69
$\text{NO}_3^-$	$\text{HNO}_3$	6.0	606	0.12	11.63	2.04	2.04

Table 2. Schedule of simulated rain events and solution pH values.

Date of Event	Solution pH by Treatment	
	pH 5.1 Treatment	pH 3.0 Treatment
1/17/92	5.1	3.0
1/23/92	5.1	3.0
1/27/92	5.1	3.0
2/17/92	5.0	2.9
2/19/92	5.0	3.0
2/24/92	5.1	3.0
2/26/92	5.1	3.0
3/02/92	5.1	3.0
3/11/92	5.1	3.0
3/17/92	5.1	3.0
3/23/92	5.1	3.0
3/27/92	4.9	3.0
4/06/92	5.1	3.0
4/13/92	5.0	3.0
4/15/92	5.1	3.0
4/21/92	5.1	3.0
4/29/92	5.1	3.0

Table 3. Example ANOVA tables for the general statistical models applied to the analyses of mature branch and seedling data.

Generalized ANOVA Table for Mature Branch and Seedling Statistical Models					
Mature Branch			Seedling		
Source	Degrees of Freedom		Source	Degrees of Freedom	
Replication	$r-1$	1	Replication	$r-1$	3
Acid Rain (A)	$a-1$	2	Acid Rain (A)	$a-1$	2
Genotype (G)	$g-1$	2	Ozone (O)	$o-1$	2
A x G	$(a-1)(g-1)$	4	A x O	$(a-1)(o-1)$	4
Error I (whole-plot)	$ag(r-1)$	9	Error I (whole-plot)	$ao(r-1)$	27
Ozone (O)	$o-1$	2	Genotype (G)	$g-1$	2
A x O	$(a-1)(o-1)$	4	A x G	$(a-1)(g-1)$	4
G x O	$(g-1)(o-1)$	4	O x G	$(o-1)(g-1)$	4
A x G x O	$(a-1)(g-1)(o-1)$	8	A x O x G	$(a-1)(o-1)(g-1)$	8
Error II (sub-plot)	$ag(o-1)(r-1)$	18	Error II (sub-plot)	$ao(g-1)(r-1)$	54

Table 4. Example RMANOVA tables for the general statistical models applied to the analyses of mature branch and seedling data.

Generalized ANOVA Table for Mature Branch and Seedling Statistical Models					
Mature Branch			Seedling		
Source	Degrees of Freedom		Source	Degrees of Freedom	
Replication	r-1	1	Replication	r-1	3
Acid Rain (A)	a-1	2	Acid Rain (A)	a-1	2
Genotype (G)	g-1	2	Ozone (O)	o-1	2
A x G	(a-1)(g-1)	4	A x O	(a-1)(o-1)	4
Error I (whole-plot)	ag(r-1)	9	Error I (whole-plot)	ao(r-1)	27
Ozone (O)	o-1	2	Genotype (G)	g-1	2
A x O	(a-1)(o-1)	4	A x G	(a-1)(g-1)	4
G x O	(g-1)(o-1)	4	O x G	(o-1)(g-1)	4
A x G x O	(a-1)(g-1)(o-1)	8	A x O x G	(a-1)(o-1)(g-1)	8
Error II (sub-plot)	ag(o-1)(r-1)	18	Error II (sub-plot)	ao(g-1)(r-1)	54
Time (M)*	m-1	8	Time (M)*	m-1	7
M x A	(m-1)(a-1)	16	M x A	(m-1)(a-1)	14
M x G	(m-1)(g-1)	16	M x O	(m-1)(o-1)	14
M x A x G	(m-1)(a-1)(g-1)	32	M x A x O	(m-1)(a-1)(o-1)	28
Error III	ag(m-1)(r-1)	72	Error III	ao(m-1)(r-1)	189
M x O	(m-1)(o-1)	16	M x G	(m-1)(g-1)	14
M x A x O	(m-1)(a-1)(o-1)	32	M x A x G	(m-1)(a-1)(g-1)	28
M x G x O	(m-1)(g-1)(o-1)	32	M x O x G	(m-1)(o-1)(g-1)	28
M x A x G x O	(m-1)(a-1)(g-1)(o-1)	64	M x A x O x G	(m-1)(a-1)(o-1)(g-1)	56
Error IV (Time)	ag(m-1)(o-1)(r-1)	144	Error IV (Time)	ao(m-1)(g-1)(r-1)	378

\* - Number of monthly measurement periods for the one-year-old (1991) foliage age-class. For the current-year (1992) foliage age-class, m-1 equals 5.

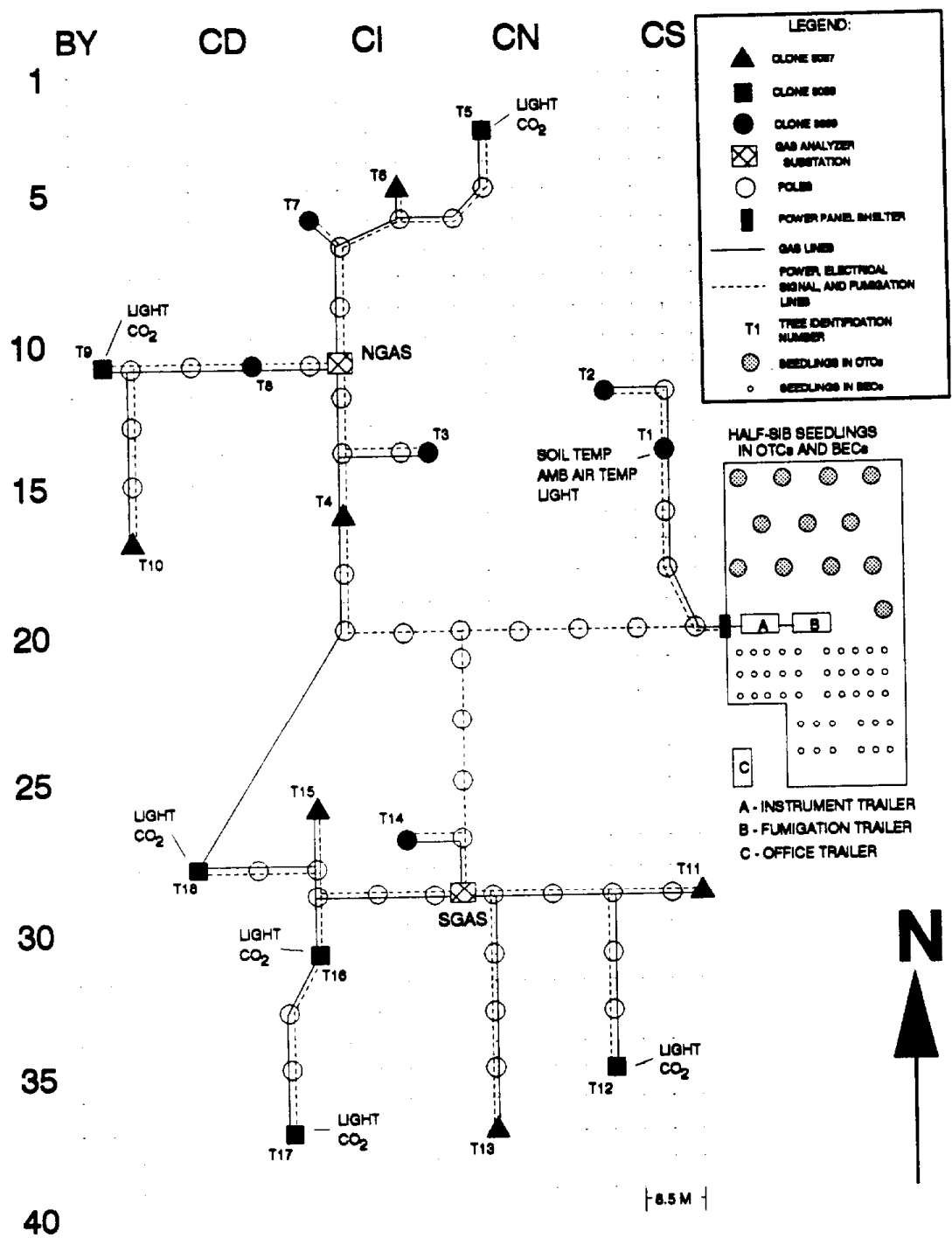


Figure 1. Layout of the controlled exposure facilities at the Chico Air Pollution And Climate Change research site (CAPACC).

## IV. Results

### A. Ozone Fumigation

Over the study period the mean ratio of ozone concentrations in the CF treatment relative to the ambient treatment was 0.53 ( $\pm 0.01$  s.e.) for tree branches and 0.58 ( $\pm 0.01$ ) for seedlings, based on 12-h average data. The mean ratio of ozone concentrations for the 2xAMB treatment relative to the AMB treatment was 1.86 ( $\pm 0.02$ ) for mature branches and 1.93 ( $\pm 0.03$ ) for seedlings. The data indicate that over the study period, treatment with elevated ozone was within 15 percent of the target ratio for mature branches and within 8 percent of the target ratio for seedlings.

Mean ambient 12-h concentration data (12 h average from 0900 to 2100 h) from September, 1991, through early December, 1992 are presented in Figure 2a. Each point represents the mean of the 12-h averages for 10 consecutive days. The vertical bars represent one standard error about the mean for each 10-day period. Ambient ozone concentration at the study site varied from lows of approximately 0.01 ppm in January to peaks ranging from 0.06 to 0.07 ppm that occurred over an extended period from May through September. Ambient ozone data collected by CARB at the Manzanita Station in Chico (located approximately 5 mi north of the study site) are presented in Figure 2a as a basis of reference.

Seasonal variation in ozone concentration as measured in the BECs is presented for mature branches and seedlings, respectively, in Figures 2b and 2c. The seasonal variation in treatment level means is similar for both life-stages. Two points deserve emphasis. First, it should be noted that ozone concentrations were not monitored at the CAPACC facility from early December, 1991, through mid-February, 1992. Thus, the linear decline in ozone concentration presented in Figures 2b and 2c for this time period do not reflect the variability that probably occurred. Secondly, the mid-summer, 1992, loss of ozone generation (June 22 to July 22) capabilities is reflected in the lack of differentiation between the AMB and 2xAMB ozone concentrations. In general, the differentiation among ozone treatment levels meet the experimental objectives in terms of relative concentration as peak values for the CF, AMB and 2xAMB, respectively, were in the ranges of 0.025 to 0.030 ppm, 0.05 to 0.06 ppm, and 0.09 to 0.11 ppm when expressed as the mean of 12-h average concentration.

The degree of variation in ozone concentration among chambers within treatment levels is better illustrated by the mean 12-h average concentrations for individual days. Such data are presented in Table 5 for five days during the 1992 study period. In general, the standard error of the mean for a treatment group is less than 1 percent of the mean regardless of the date. There is a tendency for increasing variation with increasing ambient ozone concentration. With the exception of the data for August, there is little difference in mean values for branches and seedlings subjected to the same ozone treatment. In August, seedling ozone concentrations, regardless of treatment level, appear to be approximately 10 ppb less than those for mature branches. Differentiation of ozone concentrations among treatment levels was similar among life-stages. The ratio of ozone concentrations for the CF treatment relative to the AMB treatment ranged

from 0.45 to 0.65 for seedlings and from 0.41 to 0.61 for mature branches for the five days in Table 5. The ratio of ozone concentrations for the 2xAMB treatment relative to the AMB treatment ranged from 1.47 to 2.23 for branches and from 1.49 to 2.62 for seedlings for the five days.

Diurnal patterns of ozone concentration consisted of minimum values occurring between 2300 and 0600 h followed by increases to maximums that occurred anywhere from 1100 to 2000 h. Figures 3 through 7 illustrate the diurnal variation in ozone concentration for five days during the 1992 study period. In February, there was little observed diurnal variation in ambient ozone concentration for the seedlings as values varied from about 0.025 to 0.040 ppm (Figure 3). More commonly, distinct daily peak values 0.025 to 0.060 ppm greater than the minimum occurred between 1200 and 1700 h as illustrated by the data for April, June and August (Figures 4-6). On many occasions, a second, lesser, peak concentration occurred near 2000 h (Figures 4,6-7).

Table 5. Twelve-hour (0900-2100 h) average ozone concentration for selected days over the 1992 study period by ozone treatment and life-stage. Values are means and standard errors of the 12-h averages for each treatment group.

12-Hour Ozone Concentration (ppm)							
Date	Parameter	Non-chambered Ambient	Mature Branches			Seedlings	
			CF	AMB	2xAMB	CF	AMB 2xAMB
2/16	mean	0.030	0.016	0.035	0.057	0.017	0.029
	s.e.	0.001	0.001	0.003	0.002	0.000	0.001
4/16	mean	0.030	0.017	0.026	0.068	0.018	0.030
	s.e.	0.001	0.001	0.001	0.004	0.001	0.001
6/16	mean	0.041	0.020	0.043	0.080	0.023	0.038
	s.e.	0.004	0.001	0.004	0.007	0.002	0.004
8/16	mean	0.034	0.025	0.055	0.082	0.014	0.034
	s.e.	0.003	0.002	0.004	0.007	0.001	0.003
10/16	mean	0.051	0.024	0.052	0.110	0.024	0.043
	s.e.	0.006	0.002	0.005	0.012	0.003	0.005

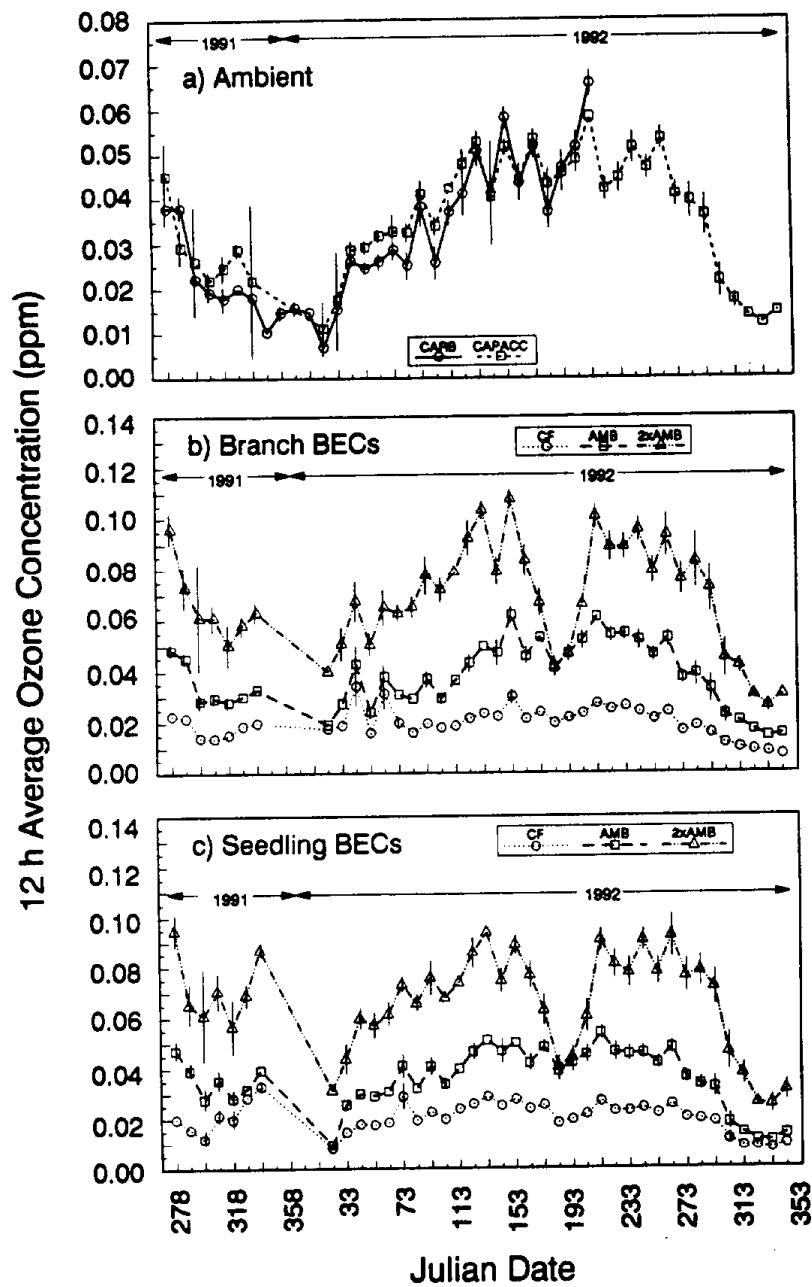


Figure 2. Seasonal variation in the 12-hour (0900-2100) average ozone concentration for a) ambient conditions sampled by CARB (Manzanita station, Chico, CA) and at the study location (CAPACC), b) mature branch BECs and c) seedling BECs.

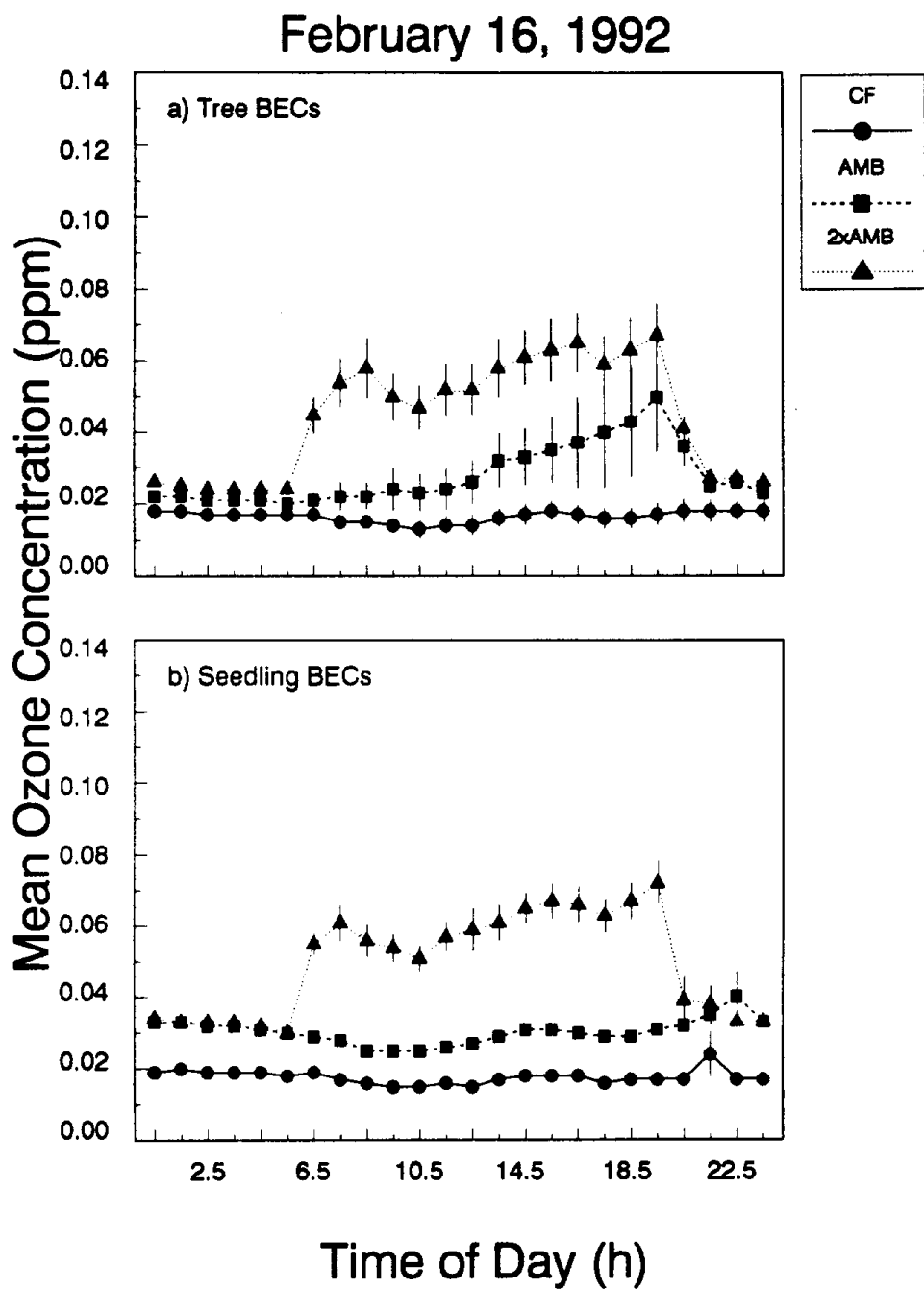


Figure 3. Diurnal variation in mean hourly ozone concentration on 16 February, 1992, for a) mature branch BECs and b) seedling BECs.

April 16, 1992

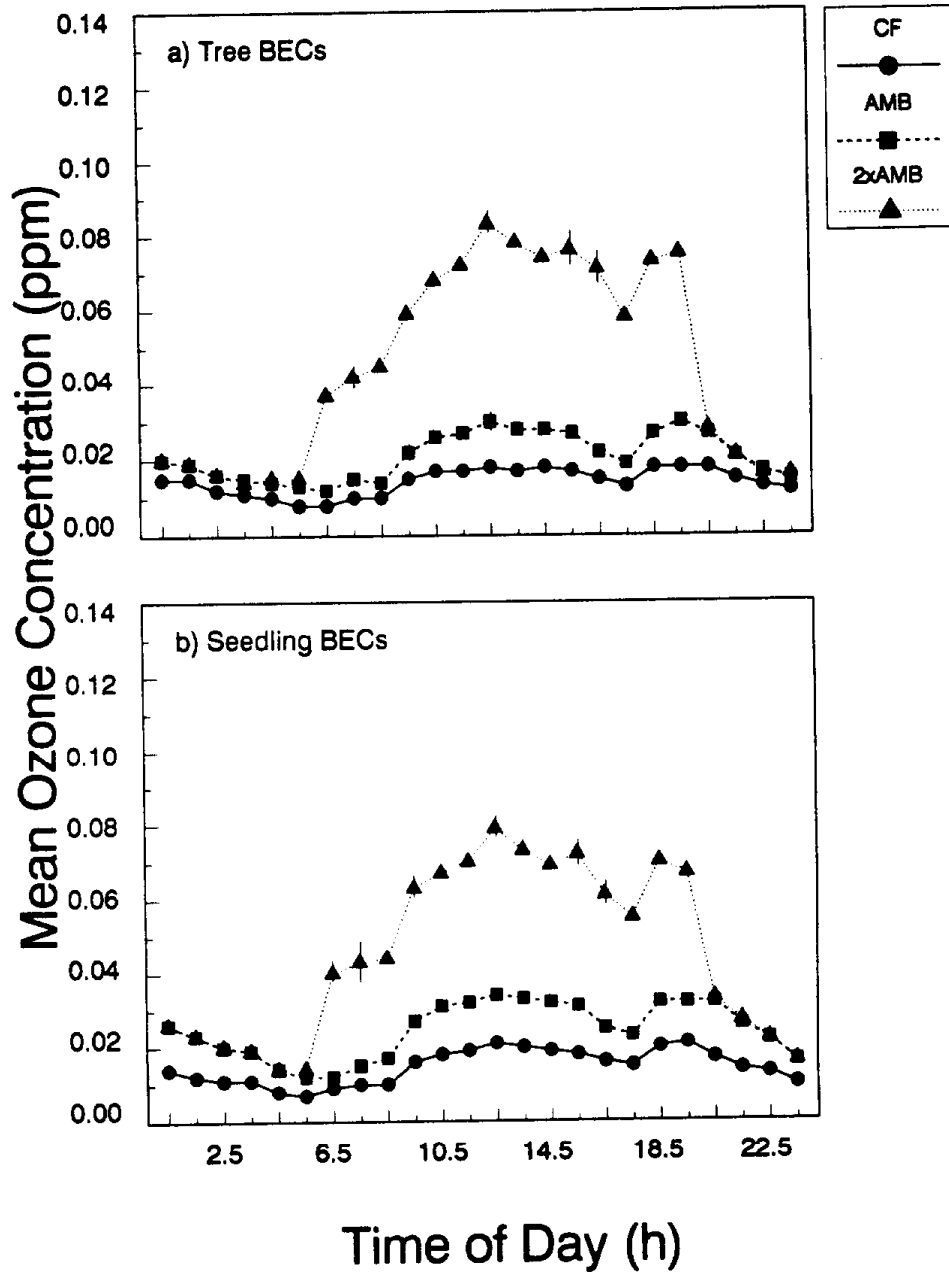


Figure 4. Diurnal variation in mean hourly ozone concentration on 16 April, 1992, for a) mature branch BECs and b) seedling BECs.

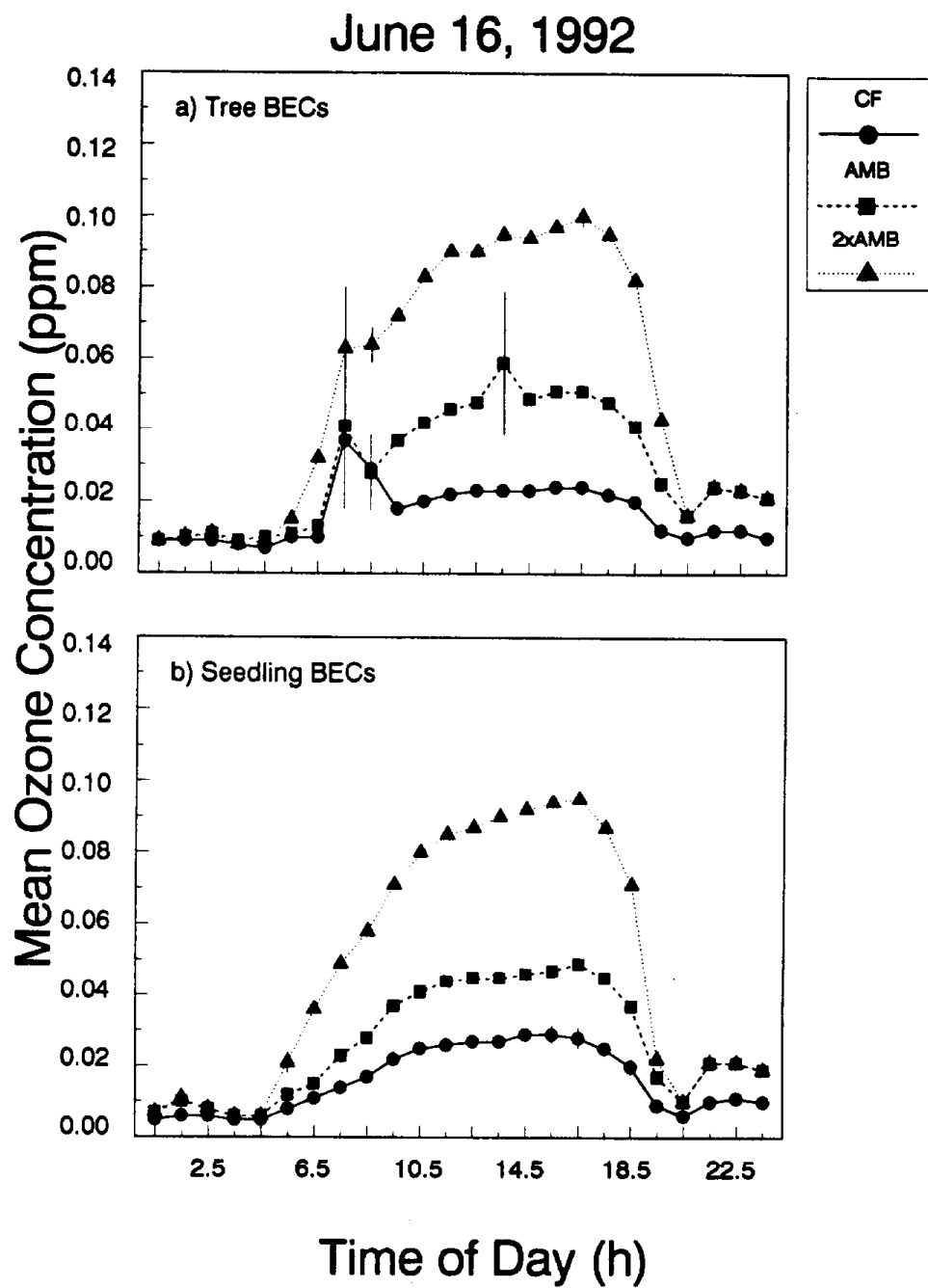


Figure 5. Diurnal variation in mean hourly ozone concentration on 16 June, 1992, for a) mature branch BECs and b) seedling BECs.

August 16, 1992

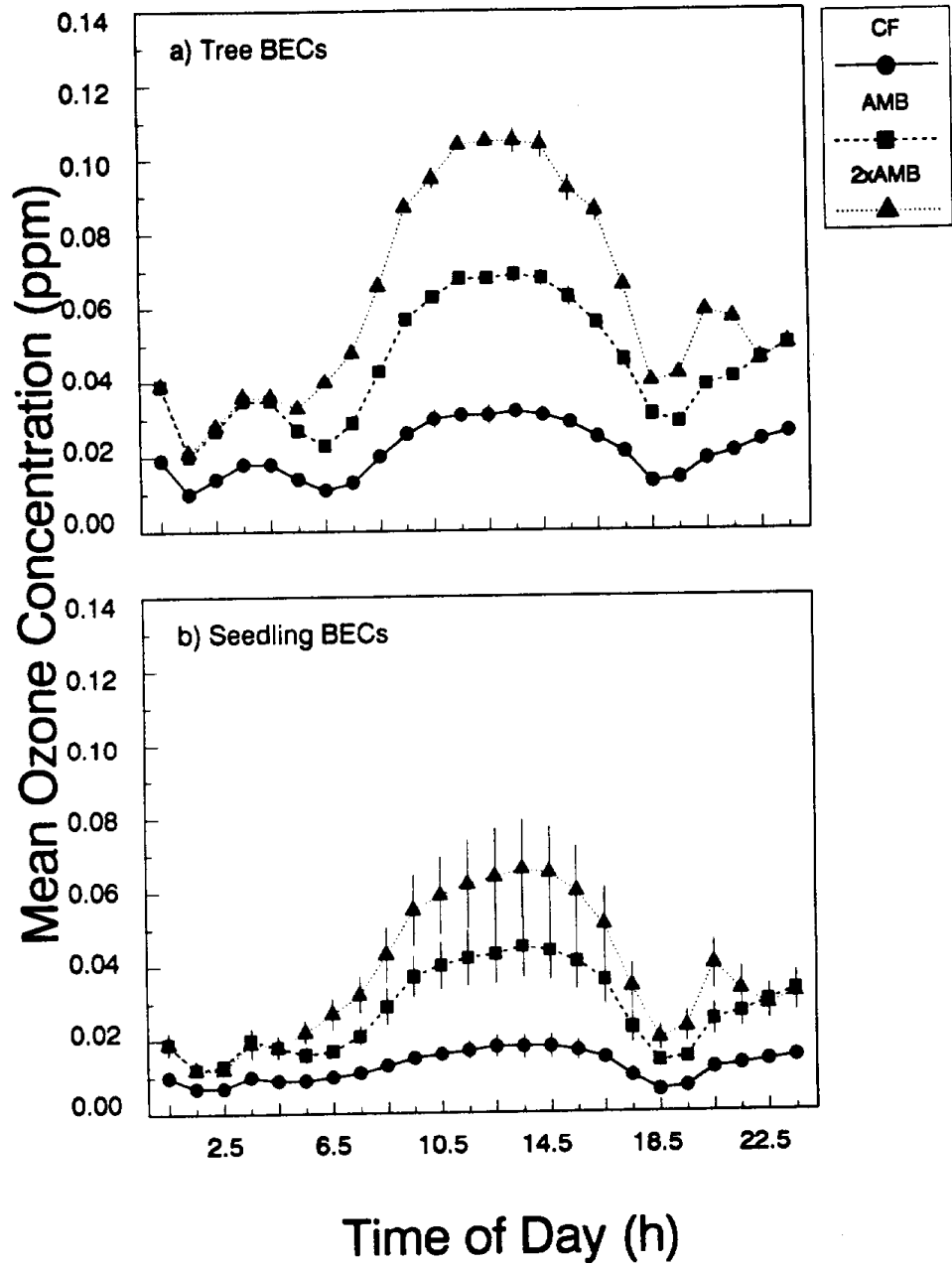


Figure 6. Diurnal variation in mean hourly ozone concentration on 16 August, 1992, for a) mature branch BECs and b) seedling BECs.

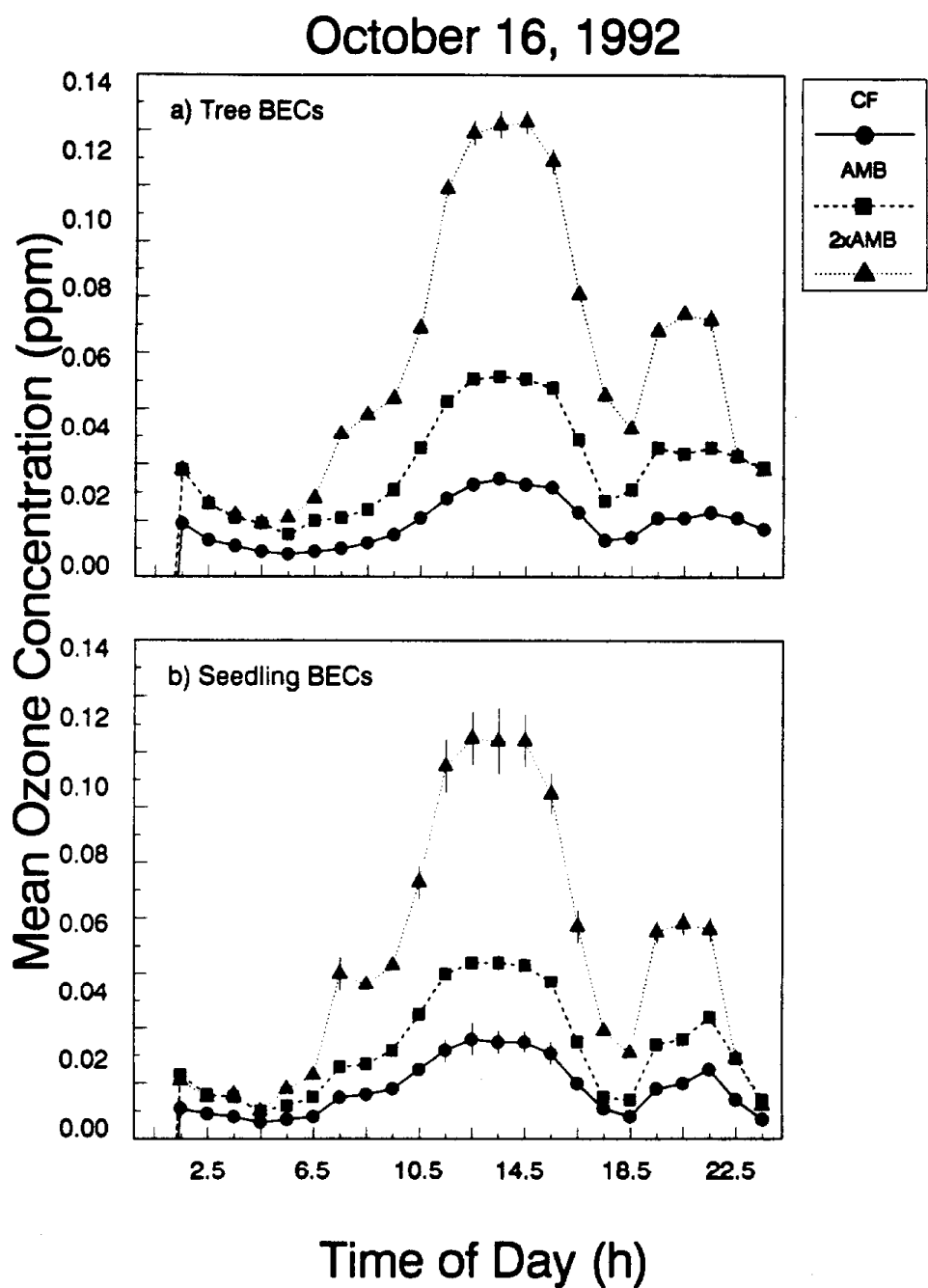


Figure 7. Diurnal variation in mean hourly ozone concentration on 16 October, 1992, for a) mature branch BECs and b) seedling BECs.

## C. Mid-day Gas Exchange

### 1. Stomatal Conductance

#### a. Seasonal pattern of conductance over all genotypes and pollutant treatments (M)

*One-year-old mature branch foliage* - When averaged over all genotypes and air pollution treatments, mean stomatal conductance ( $g_s$ ) by one-year-old mature branch foliage ranged from 0.043 to 0.074 mol m<sup>-2</sup> s<sup>-1</sup> (Figure 8) and differences over time were highly significant ( $p > 0.001$ , Table 6). The maximum mean value was observed in March while the minimum mean value occurred in August. The seasonal pattern was bimodal with a second peak occurring in July following three consecutive months of relatively stable  $g_s$  values (0.054 to 0.062 mol m<sup>-2</sup> s<sup>-1</sup>). Subsequent to the observed minimum in August, there was a substantial increase in  $g_s$  as the mean value rose to 0.067 mol m<sup>-2</sup> s<sup>-1</sup> in October.

*Current-year mature branch foliage* - Monthly measures of mid-day  $g_s$  for current-year mature branch foliage, averaged over all genotypes and air pollution treatments, ranged from 0.067 mol m<sup>-2</sup> s<sup>-1</sup> in June to 0.099 mol m<sup>-2</sup> s<sup>-1</sup> in November (Figure 8). With the exception of October, in which  $g_s$  dropped approximately 16 percent from the September value, there was a general increase in mean  $g_s$  over the study period (Figure 8). Seasonal variation in  $g_s$  of current-year mature branch foliage was very highly significant ( $p > 0.001$ , Table 6).

*One-year-old seedling foliage* - Seasonal variation in mean mid-day  $g_s$  for one-year-old seedling foliage was very highly significant ( $p > 0.001$ , Table 7) and ranged from 0.039 mol m<sup>-2</sup> s<sup>-1</sup> in February to 0.090 mol m<sup>-2</sup> s<sup>-1</sup> in both March and April (Figure 8). Two periods of substantial decline, April to June and July to October, characterize the seasonal pattern of  $g_s$ . The declines from peak values in April and July were 35 and 40 percent, respectively. There was no indication of an increase in  $g_s$  going into the month of October.

*Current-year seedling foliage* - Mid-day rates of conductance for current-year seedling foliage ranged from a maximum of 0.122 mol m<sup>-2</sup> s<sup>-1</sup> in March to a minimum of 0.108 mol m<sup>-2</sup> s<sup>-1</sup> in both September and October. The 11 percent reduction in  $g_s$  from July to September was indicative of highly significant ( $p < 0.008$ ) variation during the study period (Table 7) but there was little difference in  $g_s$  between early summer (June) and late-summer or fall (September to November) measurement periods (Figure 8).

*Lifestage and age-class comparisons* - The seasonal patterns of  $g_s$  for current-year and one-year-old foliage of seedlings and mature branches presented in Figure 8 provide strong indication of differences both between foliage age-classes and between lifestages. Conductance values for seedlings were in general greater than those for mature branches. For current-year foliage, seedling  $g_s$  values were from 15 percent to 61 percent greater

than the  $g_s$  values for mature branches. For the months of March through August,  $g_s$  values for one year-old foliage were from 8 percent to 45 percent greater for seedlings relative to mature branches.

Seasonal patterns of mid-day  $g_s$  in one-year-old foliage of seedlings and mature branches were similar for much of the study period. Values rose rapidly from February to a peak in March and subsequently fell to a low in June. Following a second peak in July,  $g_s$  again fell to low values that for branches occurred in August and for seedlings occurred in September. The major distinction between mature branch and seedling one-year-old foliage was a late season increase in  $g_s$  for the branches (a 55 percent increase from August to October) in contrast to a lack of substantial increase in  $g_s$  for seedling one-year-old foliage.

Two differences distinguish the seasonal  $g_s$  patterns for seedling and mature branch current-year foliage. First, from August to September,  $g_s$  values increased 15 percent for mature branches while  $g_s$  values for seedlings declined 8 percent (Figure 8). Secondly, from September to October,  $g_s$  values for mature branches declined 16 percent, while  $g_s$  values for seedlings were unchanged.

As indicated above, statistical comparisons among foliage age-classes were not possible due to confounding with sampling date and statistical comparisons between life-stages were not performed due to differences in the nesting of factors in the treatment structure of the experiment. Yet, the mean and standard error estimates presented in Figure 8, suggest that the differences in  $g_s$  between lifestages and between foliage age-classes are likely to be real and significant.

#### b. Chamber effect on the seasonal pattern of conductance

*One-year-old mature branch foliage* - Monthly mid-day  $g_s$  values for one-year-old foliage of non-chambered mature branches (NCAMB) ranged from  $0.037 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.083 \text{ mol m}^{-2} \text{ s}^{-1}$  (Figure 9a). Monthly  $g_s$  values for chambered branches exposed to ambient air (AMB, averaged over all genotypes and acid rain treatments) ranged from  $0.043 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.081 \text{ mol m}^{-2} \text{ s}^{-1}$  (Figure 9a). For both AMB and NCAMB branches, peak values occurred in March and July while minimum values occurred in February and August (Figure 9a). Relative to AMB values,  $g_s$  values for NCAMB branches differed by 4 to 19 percent with the greatest differences occurring in February and October (19 and 16 percent, respectively). During February, May, and July through October,  $g_s$  values were greater for AMB branches while in March, April and June values were greater for NCAMB branches. With the possible exception of February and October, differences in  $g_s$  of one-year-old foliage between AMB and NCAMB branches were not statistically significant.

*Current-year mature branch foliage* - Stomatal conductance values for current-year foliage ranged from  $0.064 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.100 \text{ mol m}^{-2} \text{ s}^{-1}$  for AMB branches and from  $0.067 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.101 \text{ mol m}^{-2} \text{ s}^{-1}$  for NCAMB branches (Figure 9a). Seasonal

patterns for both AMB and NCAMB branches were similar and differences in  $g_s$  ranged from 3 to 11 percent (Figure 9a). Conductance values of current-year foliage were greater for NCAMB, relative to AMB, in June, July, September and October (Figure 9a).

*One-year-old seedling foliage* - Seasonal patterns of mid-day  $g_s$  for AMB and NCAMB one-year-old seedling foliage were similar although  $g_s$  values for NCAMB were significantly greater than those for AMB seedlings in the months of April and May (Figure 9b). For the subsequent months, differences in  $g_s$  between NCAMB and AMB, relative to AMB, ranged from 6 percent to 20 percent.

*Current-year seedling foliage* - From July through November,  $g_s$  values for current-year foliage were greater for AMB seedlings than for NCAMB seedlings (Figure 9b). Values ranged from  $0.103 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.118 \text{ mol m}^{-2} \text{ s}^{-1}$  for AMB seedlings and from  $0.103 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.107 \text{ mol m}^{-2} \text{ s}^{-1}$  for NCAMB (Figure 9b). The difference in  $g_s$  between AMB and NCAMB treatments was greatest in the months of August and November (12 and 15 percent, respectively). The only substantial difference in seasonal patterns between the AMB and NCAMB treatments was evident in October and November as  $g_s$  increased for AMB seedlings and decreased for NCAMB seedlings (Figure 9b).

*Lifestage and age-class comparisons* - Evaluation of the data presented in Figure 9a indicate that age-class differences in  $g_s$  response to chamber enclosure were absent for mature branch foliage. For seedlings, chamber effects were present in the spring for one-year-old foliage and in mid-summer and early-fall for current-year foliage (Figure 9b). Current-year foliage  $g_s$  values were greater for AMB seedlings while for one-year-old foliage, the values for NCAMB seedlings were greater than the values for AMB seedlings (Figure 9b).

#### c. Genotype effect (G)

*One-year-old mature branch foliage* - When averaged over all measurement dates and pollutant treatments, stomatal  $g_s$  values of one-year-old mature branch tissue tended to be greater for genotype 3087 than for either genotype 3088 or genotype 3399 (Table 8). Although  $g_s$  values for genotype 3087 were 5.2 percent and 5.0 percent greater than those for genotypes 3088 and 3399, respectively, the differences among the three genotypes were not statistically significant.

*Current-year mature branch foliage* - Mean mid-day  $g_s$  for current-year mature branch foliage ranged from  $0.081 \text{ mol m}^{-2} \text{ s}^{-1}$  for genotype 3087 to  $0.087 \text{ mol m}^{-2} \text{ s}^{-1}$  for genotype 3088 (Table 8). Among genotype differences in  $g_s$  were not statistically significant at the  $p=0.05$  level.

*One-year-old seedling foliage* - Mean values of  $g_s$ , calculated over all measurement dates and pollutant treatments for one-year-old seedling foliage were  $0.063 \text{ mol m}^{-2} \text{ s}^{-1}$  for genotype 3087,  $0.069 \text{ mol m}^{-2} \text{ s}^{-1}$  for genotype 3088 and  $0.065 \text{ mol m}^{-2} \text{ s}^{-1}$  for genotype 3399 (Table 8). The mean values for genotype 3088 were significantly greater ( $p < 0.05$ , Tukey's HSD) than those of genotype 3087. The differences in mean  $g_s$  values between genotypes 3087 and 3399 and between genotypes 3088 and 3399 were not statistically significant.

*Current-year seedling foliage* - Mean  $g_s$  values of current-year seedling foliage for genotypes 3088 and 3399 tended to be greater than those for genotype 3087. Mean  $g_s$  values were  $0.108$ ,  $0.114$  and  $0.117 \text{ mol m}^{-2} \text{ s}^{-1}$  for the half-sib seedlings of genotypes 3087, 3088 and 3399, respectively (Table 8). Despite having an 8 percent greater value, the mean  $g_s$  for genotype 3399 was not significantly greater than that for genotype 3087.

*Lifestage and age-class comparisons* - Few distinct trends can be identified in comparison of the genotype responses between lifestages or foliage age-classes. For current-year tissue of both age-classes, there was a tendency for the lowest  $g_s$  values to be associated with genotype 3087 (Table 8). For one year-old foliage, the ranking of genotype means differed between mature branches and seedlings (Table 8).

Relative to mature branch values, mean  $g_s$  values of current-year foliage for seedlings were 34, 31 and 40 percent greater for clones 3087, 3088 and 3399, respectively. Among genotypes, differences between seedling and mature branch  $g_s$  for one-year-old foliage were 0.6, 16, and 9 percent for genotypes 3087, 3088 and 3399, respectively.

#### d. Seasonal variation in genotype effect (M x G)

*One-year-old mature branch foliage* - Although a significant genotype main effect was not observed for one-year-old mature branch foliage, there was a nearly significant ( $p = 0.054$ ) month x genotype interaction effect on  $g_s$  (Table 6). The nature of the interaction is illustrated in Figure 10a. For most of the study period (April through October),  $g_s$  values of one-year-old mature branch foliage were greatest for genotype 3087 and followed closely by values for 3399. From April through August, the lowest mean values of  $g_s$  were observed for clone 3088. Mean values for genotype 3088 deviated substantially from those of the other genotypes during the months of March, May and August. In March,  $g_s$  for genotype 3088 was 37 percent greater than that of genotype 3087. In May and August, mean  $g_s$  values for genotype 3088 were 32 percent and 43 percent, respectively, less than those of genotype 3087. By September, there were no differences in  $g_s$  among the three genotypes. In summary, one-year-old mature branch foliage in 3088 had substantially greater seasonal amplitude in  $g_s$  than did corresponding foliage of the 3087 and 3399 genotypes.

*Current-year mature branch foliage* - Mean  $g_s$  values for current-year mature branch foliage demonstrated a tendency to increase over the study period from 0.060 to 0.088  $\text{mol m}^{-2} \text{s}^{-1}$ , 0.073 to 0.110  $\text{mol m}^{-2} \text{s}^{-1}$  and 0.069 to 0.101  $\text{mol m}^{-2} \text{s}^{-1}$  for genotypes 3087, 3088 and 3399, respectively (Figure 10a). With the exceptions of July and August, the greatest mean values were observed for genotype 3088. In July, there was little difference in  $g_s$  among genotypes while in August, the mean  $g_s$  value for genotype 3088 was 15 percent and 17 percent less than the mean values for genotypes 3087 and 3399, respectively. Although differences in mean  $g_s$  values among genotypes were as large as 25 percent (genotype 3088 versus genotype 3087 in November, Figure 10a), the month  $\times$  genotype interaction was not statistically significant at the  $p=0.05$  level (Table 6).

*One-year-old seedling foliage* - Seasonal patterns of mean  $g_s$  for one-year-old seedling foliage were similar for all three genotypes (Figure 10b). There was a bimodal pattern with peak values occurring in April and July. The greatest mean  $g_s$  values were observed for genotype 3088 in the months of March through June and August through October. The lowest  $g_s$  values were commonly observed for genotype 3087 (February through June, September and October). Within any month the range of mean values among genotypes was less than 0.011  $\text{mol m}^{-2} \text{s}^{-1}$ . There was no statistically significant month  $\times$  genotype interaction (Table 7).

*Current-year seedling foliage* - Seasonal variation in  $g_s$  for current-year seedling foliage was similar for genotypes 3088 and 3399 (Figure 10b). Peak values occurred in July for genotype 3088 and in August for genotype 3399 and were followed by slight reductions to lows in September and October, respectively. Both genotypes 3088 and 3399 demonstrated a slight increase in  $g_s$  from October to November. The mean  $g_s$  values ranged from 0.108  $\text{mol m}^{-2} \text{s}^{-1}$  to 0.119  $\text{mol m}^{-2} \text{s}^{-1}$  for genotype 3088 and from 0.107  $\text{mol m}^{-2} \text{s}^{-1}$  to 0.121  $\text{mol m}^{-2} \text{s}^{-1}$  for genotype 3399 (Figure 10b). In contrast, the seasonal  $g_s$  amplitude for current-year seedling foliage of genotype 3087 was 0.030  $\text{mol m}^{-2} \text{s}^{-1}$  and ranged from 0.096  $\text{mol m}^{-2} \text{s}^{-1}$  to 0.126  $\text{mol m}^{-2} \text{s}^{-1}$  (Figure 10b). In June, mean  $g_s$  for genotype 3087 was 16 percent and 18 percent less than the mean  $g_s$  for genotypes 3088 and 3399, respectively. Although genotype 3087 also had the lowest  $g_s$  during the months of August through November, the difference in  $g_s$  among genotypes was only substantial during June. The month  $\times$  genotype interaction effect was nearly significant ( $p=0.051$ , Table 7).

*Lifestage and age-class comparisons* - Subjective comparison of the month  $\times$  genotype  $g_s$  means indicates few trends. One interesting feature of the data presented in Figures 10a and b is that the relative difference in  $g_s$  values between current-year and one-year-old foliage was greater for seedlings than for mature branches. There were also differences between lifestages and between foliage age-classes in the relative ranking of  $g_s$  by genotype. For example, in comparing one-year-old foliage, genotype 3088 tended to have the highest  $g_s$  rates for seedlings while genotype 3087 tended to have the highest  $g_s$  values for mature branches. For current-year foliage, genotype 3399 tended to have the highest  $g_s$  values for seedlings while genotype 3088 tended to have the highest  $g_s$  values

for mature branches. In general, there was much greater variation in seasonal  $g_s$  patterns among genotypes for mature branches than for seedlings and generally, the increased variability was due to deviation of one genotype from the other two (genotype 3088 for one-year-old foliage and genotype 3087 for current-year foliage).

e. Acidic rain effect (A)

*One-year-old mature branch foliage* - Treatment mean  $g_s$  values, averaged over all measurement dates, genotypes and ozone treatments were  $0.059 \text{ mol m}^{-2} \text{ s}^{-1}$ ,  $0.061 \text{ mol m}^{-2} \text{ s}^{-1}$ , and  $0.062 \text{ mol m}^{-2} \text{ s}^{-1}$  for one year-old mature branch tissue exposed to no acid rain (NAP), pH 5.1 simulated rain (pH 5.1) and pH 3.0 simulated rain (pH 3.0), respectively (Table 9). Differences among treatment means were not significant at the  $p=0.05$  level of significance (Table 6).

*Current-year mature branch foliage* - Current-year mature branch  $g_s$  was  $0.084 \text{ mol m}^{-2} \text{ s}^{-1}$ ,  $0.088 \text{ mol m}^{-2} \text{ s}^{-1}$ , and  $0.079 \text{ mol m}^{-2} \text{ s}^{-1}$  for tissues receiving the NAP, pH 5.1 and pH 3.0 acid rain treatments, respectively (Table 9). Although the mean  $g_s$  for foliage in the pH 3.0 treatment was 6 percent and 11 percent less than the means for foliage receiving the NAP and pH 5.1 treatments, respectively, differences among acid rain treatments were not significant at the  $p=0.05$  level (Table 6).

*One-year-old seedling foliage* - Mean  $g_s$  for one-year-old seedling foliage exposed to NAP, pH 5.1 and pH 3.0 treatments was, respectively,  $0.066 \text{ mol m}^{-2} \text{ s}^{-1}$ ,  $0.065 \text{ mol m}^{-2} \text{ s}^{-1}$  and  $0.067 \text{ mol m}^{-2} \text{ s}^{-1}$ , when averaged over all measurement dates, genotypes and ozone treatments (Table 9). Differences among acid rain treatment level means were not statistically significant (Table 7).

*Current-year seedling foliage* - Mean  $g_s$  for current-year seedling foliage was substantially greater for tissue exposed to pH 5.1 rain than for corresponding tissue exposed to NAP or pH 3.0 rain (Table 9). The mean value for the pH 5.1 treatment was 10 percent and 12 percent greater than the mean values for the NAP and pH 3.0 treatments, respectively. This apparent acid rain main effect was nearly significant ( $p=0.097$ , Table 7) but Tukey's HSD mean separation test indicated that differences among treatment means were not significant at the  $p=0.05$  level.

*Lifestage and age-class comparisons* - Although strong effects of acid rain exposure are not present in the data, two trends are apparent in the responses by different foliage age-classes and lifestages. For one-year-old foliage, the highest conductance values were observed for those branches and seedlings receiving pH 3.0 simulated rain (Table 9). For current-year foliage,  $g_s$  values were greatest for the pH 5.1 treatment, and lowest for the pH 3.0 treatment, regardless of lifestage (Table 9).

f. Seasonal variation in acidic rain effect (A x M)

*One-year-old mature branch foliage* - Among measurement dates, mean  $g_s$  for one-year-old mature branch foliage ranged from  $0.041 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.078 \text{ mol m}^{-2} \text{ s}^{-1}$  when exposed to NAP. For branches exposed to pH 5.1 or pH 3.0, the observed ranges were  $0.051 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.074 \text{ mol m}^{-2} \text{ s}^{-1}$ , and from  $0.044 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.074 \text{ mol m}^{-2} \text{ s}^{-1}$ , respectively (Figure 11a). The seasonal pattern of  $g_s$  was similar for all three treatments. Peak values were observed in March and July and following the occurrence of minimum values in August there was a late season increase in mean  $g_s$  (Figure 11a). Differences among treatment level means were small for any given month with the range being from  $0.003 \text{ mol m}^{-2} \text{ s}^{-1}$  in August to  $0.010 \text{ mol m}^{-2} \text{ s}^{-1}$  in May. In general,  $g_s$  was lowest for the NAP treatment. Maximum  $g_s$  was observed in April through June for the pH 3.0 treatment and  $g_s$  values for the pH 5.1 and the pH 3.0 treatments were virtually identical and occurred from August through October (Figure 11a). The month x acid rain interaction effect was not significant at the  $p=0.05$  level (Table 6).

*Current-year mature branch foliage* - Monthly mid-day  $g_s$  for current-year mature branch foliage ranged from  $0.066 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.107 \text{ mol m}^{-2} \text{ s}^{-1}$  for the NAP treatment, from  $0.070 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.102 \text{ mol m}^{-2} \text{ s}^{-1}$  for the pH 5.1 treatment and from  $0.066 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.089 \text{ mol m}^{-2} \text{ s}^{-1}$  for the pH 3.0 treatment (Figure 11a). From June through September, the greatest mean values were observed for the pH 5.1 treatment. Branches receiving the pH 3.0 had the lowest  $g_s$  in every month with the exception of October, at which time there was no difference in  $g_s$  among the three treatments. The greatest differences among treatment means occurred in August and November but these differences were not significant and there was no significant month x acid rain interaction (Table 6).

*One-year-old seedling foliage* - One-year-old seedling foliage demonstrated a bimodal seasonal pattern of  $g_s$  regardless of acid rain treatment (Figure 11b). Early season peak values occurred in either March (NAP, pH 5.1) or in April (pH 3.0). For all three treatments, seasonal low values occurred in June followed by a second peak in July. The observed ranges in  $g_s$  for the NAP, pH 5.1 and pH 3.0 treatments were, respectively, from  $0.039 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.085 \text{ mol m}^{-2} \text{ s}^{-1}$ , from  $0.033 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.090 \text{ mol m}^{-2} \text{ s}^{-1}$ , and from  $0.046 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.102 \text{ mol m}^{-2} \text{ s}^{-1}$  (Figure 11b). Seedlings subjected to the pH 3.0 treatment had the greatest  $g_s$  values during the early season (February through April) and the lowest values at the end of the season (September and October). For individual months the maximum difference among treatment level means varied from  $0.005 \text{ mol m}^{-2} \text{ s}^{-1}$  in June to  $0.027 \text{ mol m}^{-2} \text{ s}^{-1}$  in March. There was no significant month x acid rain interaction for one-year-old seedling foliage (Table 7).

*Current-year seedling foliage* - There was a significant month x acid rain interaction ( $p=0.001$ ) effect on monthly  $g_s$  for current-year foliage of seedlings (Table 7). This interaction was manifest as a distinct difference in the seasonal pattern of mid-day  $g_s$  between seedlings receiving the pH 5.1 treatment and those receiving either the NAP or

pH 3.1 treatments. There was a large seasonal amplitude in  $g_s$  for the pH 5.1 treatment as mean values ranged from  $0.104 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.127 \text{ mol m}^{-2} \text{ s}^{-1}$ . Seedlings exposed to pH 5.1 had a distinct peak in July, followed by a continuous decline to a low in November (Figure 11b). In contrast, seedlings exposed both to NAP and pH 3.0 treatments had more limited seasonal amplitude as monthly mean  $g_s$  ranged from  $0.107 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.114 \text{ mol m}^{-2} \text{ s}^{-1}$  and from  $0.098 \text{ mol m}^{-2} \text{ s}^{-1}$  to  $0.114 \text{ mol m}^{-2} \text{ s}^{-1}$ , respectively (Figure 11b). The greatest differences among treatment means were present in July as the mean value for the pH 5.1 treatment was 33 percent and 47 percent greater than the values for the NAP and pH 3.0 treatments, respectively.

*Lifestage and foliage age-class comparisons* - There was a tendency during the summer months for current-year foliage exposed to pH 5.1 rain to have higher  $g_s$  relative to corresponding foliage exposed to either NAP or pH 3.0 rain. By September, this tendency was absent and there was substantial variation between seedlings and branches in the response of current-year foliage to the different acid rain treatments (Figures 11a and b).

For one-year-old foliage of mature branches, the apparent  $g_s$  response to the acid rain treatments noted for seedlings was absent. Specifically, the trend for a late-season decline in  $g_s$  under the pH 3.0 treatment observed for seedlings was not observed for mature branches. Also, the early season tendency for  $g_s$  values to be greatest under the pH 3.0 treatment was observed for one-year-old foliage of seedlings but not for mature branches (Figures 11a and b).

#### g. Interactive effect of acidic rain and genotype (A x G)

*One-year-old mature branch foliage* - Mean  $g_s$  for one-year-old branch foliage exposed to NAP ranged among genotypes from  $0.055 \text{ mol m}^{-2} \text{ s}^{-1}$  for 3087 to  $0.0613 \text{ mol m}^{-2} \text{ s}^{-1}$  for 3399 (Table 10). Mean values for corresponding foliage exposed to pH 5.1 rain, ranged from  $0.058 \text{ mol m}^{-2} \text{ s}^{-1}$  for 3399 to  $0.063 \text{ mol m}^{-2} \text{ s}^{-1}$  for 3087 (Table 10). Tissues exposed to pH 3.0 had mean  $g_s$  ranging from  $0.058 \text{ mol m}^{-2} \text{ s}^{-1}$  for 3088 to  $0.069 \text{ mol m}^{-2} \text{ s}^{-1}$  for 3087 (Table 10). For each genotype there was a unique trend in  $g_s$  response to acid rain. For genotype 3087,  $g_s$  increased with increasing acidity exposure (Table 10). For genotype 3088, the greatest  $g_s$  was observed for the pH 5.1 treatment and the lowest for the NAP treatment (Table 10). For genotype 3399, the NAP treatment had the greatest  $g_s$  and there was virtually no difference in  $g_s$  between the two simulated rain treatments. None of the differences among acidic rain x genotype means was significant at the  $p=0.05$  level.

*Current-year mature branch foliage* - As with older mature branch foliage, there was no significant acid rain x genotype interaction ( $p=0.05$ ) for current-year mature branch foliage (Table 6). For genotypes 3088 and 3399, the observed mean  $g_s$  values were greatest for the pH 5.1 treatment ( $0.091$  and  $0.092 \text{ mol m}^{-2} \text{ s}^{-1}$ , respectively) and lowest for the pH 3.0 treatment ( $0.082$  and  $0.073$ , respectively; Table 10). For genotype 3087, the mean  $g_s$  was lowest for the NAP treatment ( $0.076 \text{ mol m}^{-2} \text{ s}^{-1}$ ) and there was little

difference in mean  $g_s$  between the pH 5.1 and the pH 3.0 treatments ( $0.083 \text{ mol m}^{-2} \text{ s}^{-1}$  for both; Table 10).

*One-year-old seedling foliage* - For seedlings of genotype 3087, there was little difference in mean  $g_s$  for one-year-old foliage among acid rain treatments as values ranged from  $0.061 \text{ mol m}^{-2} \text{ s}^{-1}$  for the pH 5.1 treatment to  $0.064 \text{ mol m}^{-2} \text{ s}^{-1}$  for the pH 3.0 treatment 3088 (Table 10). Among acid rain treatments, mean values for genotype 3088 seedlings ranged from  $0.067 \text{ mol m}^{-2} \text{ s}^{-1}$  at pH 5.1 to  $0.072$  at pH 3.0 (Table 10). The range in treatment level means for genotype 3399 seedlings was from  $0.064 \text{ mol m}^{-2} \text{ s}^{-1}$  at both NAP and pH 3.0 to  $0.066 \text{ mol m}^{-2} \text{ s}^{-1}$  at pH 5.1 (Table 10). Within genotype differences in mean  $g_s$  among acid rain treatments were negligible and that differences among genotypes in the relative ranking of acid rain treatment means were not substantial. As indicated in Table 7, there was no significant acid rain x genotype effect on  $g_s$  for one-year-old seedling foliage.

*Current-year seedling foliage* - For all three genotypes, mean  $g_s$  by current-year seedling foliage was greatest in the pH 5.1 treatment (Table 10). For genotypes 3087 and 3399, there were negligible  $g_s$  differences between the NAP and pH 3.0 treatments (Table 10). For genotype 3088,  $g_s$  for the NAP treatment was slightly greater than that for the pH 3.0 treatment (Table 10). Although the RMANOVA failed to indicate a significant acid rain x genotype effect ( $p=0.617$ ; Table 8), application of Tukey's HSD mean separation test indicated that the mean  $g_s$  value for genotype 3399 exposed to pH 5.1 rain was significantly greater ( $p<0.05$ ) than the mean  $g_s$  values for genotype 3087 exposed to NAP and all genotypes exposed to pH 3.0 rain.

*Lifestage and foliage age-class comparisons* - For current-year foliage of both branches and seedlings, the most common trend was for the highest mean  $g_s$  values to be associated with the pH 5.1 rain treatment with  $g_s$  for the NAP treatment to be either greater than or nearly equal to that for the pH 3.0 treatment (Table 10). Consistent trends in  $g_s$  with respect to genotype and acid rain treatment are lacking for one-year-old foliage of both mature branches and seedlings. Inspection of the data for one-year-old branch foliage in Table 10 reveals further that the apparent trend for highest  $g_s$  values to be associated with the pH 3.0 treatment (as noted in section e.), is due primarily to the relatively high  $g_s$  value ( $0.072 \text{ mol m}^{-2} \text{ s}^{-1}$ ) for genotype 3088 exposed to pH 3.0. One-year-old foliage of both 3087 and 3399 fail to demonstrate any trend for increased  $g_s$  with exposure to pH 3.0 rain.

#### h. Acidic rain application effect

The potential effect of acid rain application on  $g_s$  was analyzed by comparing mean values (over all measurement dates) for seedlings and branches exposed to ambient air (AMB) and either the NAP or pH 5.1 acid rain treatments. This approach eliminates potential confounding that may arise from inclusion of the CF and 2xAMB ozone treatment effects and the acidity and nutrient effects of the pH 3.0 treatment.

*One-year-old mature branch foliage* - One-year-old foliage of mature branches exposed to ambient ozone concentration had a mean  $g_s$  of  $0.059 \text{ mol m}^{-2} \text{ s}^{-1}$  when exposed to NAP and  $0.065 \text{ mol m}^{-2} \text{ s}^{-1}$  when exposed to pH 5.1 rain. The 10 percent difference in  $g_s$  between rain-exposed and non-exposed tissue was not statistically significant at the  $p=0.05$  level of significance (Table 11).

*Current-year mature branch foliage* - The mean  $g_s$  values for current-year mature branch foliage were  $0.083 \text{ mol m}^{-2} \text{ s}^{-1}$  and  $0.088 \text{ mol m}^{-2} \text{ s}^{-1}$  for the NAP and pH 5.1 treatments, respectively. The difference in mean  $g_s$  between the rain and no-rain treatments was not significant at the  $p=0.05$  level (Table 11).

*One-year-old seedling foliage* - There was no statistical difference ( $p=0.05$ ) in  $g_s$  between one-year-old seedling foliage exposed to pH 5.1 rain relative to that not exposed to simulated rain (Table 11). Mean  $g_s$  values for the NAP and pH 5.1 treatments were  $0.068 \text{ mol m}^{-2} \text{ s}^{-1}$  and  $0.067 \text{ mol m}^{-2} \text{ s}^{-1}$  (Table 11).

*Current-year seedling foliage* - Current-year seedling foliage  $g_s$  values were  $0.112 \text{ mol m}^{-2} \text{ s}^{-1}$  for tissue receiving the NAP treatment and  $0.121 \text{ mol m}^{-2} \text{ s}^{-1}$  for tissue receiving the pH 5.1 treatment (Table 11). The 8 percent difference in the treatment means was not statistically significant.

*Lifestage and foliage age-class comparisons* - With the exception of one-year-old seedling foliage, foliage exposed to pH 5.1 simulated rain had  $g_s$  values greater than tissues receiving no simulated rain. The extent of this difference ranged from 5 to 9 percent and was not statistically significant. For one-year-old seedling foliage, there was virtually no difference between mean  $g_s$  values.

*Comparison of rain application effect to acidic rain effect* - The  $g_s$  difference between tissues exposed to NAP and pH 5.1 ranged from 1.5 percent to 8.9 percent, depending on foliage age-class and lifestage. In comparison, maximum differences among mean  $g_s$  values for the acid rain main effects presented in Table 9 ranged from 3 to 12 percent, depending on foliage age-class and lifestage. The similar magnitude of the differences among treatment level means suggests that the effect of rain application may have as much effect on  $g_s$  variation as the influence of rainfall acidity, either individually or in interaction with various ozone levels and measurement dates. The absence of significant acid rain main and interactive effects in the RMANOVA (Tables 6 and 7) indicates that the rain application effect did not lead to an incorrect conclusion that significant rainfall acidity effects were present.

i. Ozone effect (O)

*One-year-old mature branch foliage* - Stomatal conductance by one-year-old foliage of mature branches decreased with elevated ozone exposure. Mean values (calculated over all genotypes, acid rain treatments and months) for the CF, AMB and 2xAMB

treatments were, respectively, 0.064, 0.063 and 0.054 mol m<sup>-2</sup> s<sup>-1</sup> (Table 12). The effect of ozone was highly significant (p=0.001) as the mean values for the CF and AMB treatments were significantly greater than the mean value for the 2xAMB ozone treatment (Tables 6 and 12).

*Current-year mature branch foliage* - Mean  $g_s$  by current-year branch foliage decreased with increasing ozone exposure but the effect of ozone was not statistically significant at the p=0.05 level (Table 6). Mean  $g_s$  was 0.088, 0.084 and 0.081 mol m<sup>-2</sup> s<sup>-1</sup> for the CF, AMB and 2xAMB treatments, respectively (Table 12).

*One-year-old seedling foliage* - One-year-old seedling foliage demonstrated decreased  $g_s$  with increasing ozone exposure (Table 12). Mean  $g_s$  varied from 0.061 mol m<sup>-2</sup> s<sup>-1</sup> for the 2xAMB treatment to 0.070 mol m<sup>-2</sup> s<sup>-1</sup> for the CF treatment. Differences among treatment level means were not statistically significant at the p=0.05 level (Table 7).

*Current-year seedling foliage* - There was a trend for decreased  $g_s$  with increasing ozone exposure for current-year foliage of seedlings (Table 12). Mean  $g_s$  for the CF, AMB and 2xAMB treatments was, respectively, 0.120, 0.113 and 0.106 mol m<sup>-2</sup> s<sup>-1</sup>. The effect of ozone exposure on  $g_s$  was not significant at the p=0.05 level (Table 7).

*Lifestage and foliage age-class comparisons* - The trend for reduced  $g_s$  with increasing ozone exposure was common to all foliage age classes and plant lifestages (Table 12). Differences in the degree of reduction among tissue types is reflected in the variation in the significance of ozone effect. The percent decrease in  $g_s$  for the 2xAMB treatment, relative to the AMB treatment (apparent ozone decrease), ranged from 4 percent for current-year mature branch foliage to 13 percent for one-year-old mature branch foliage. For both seedlings and branches, the percent decrease in  $g_s$  for the 2xAMB treatment was greater for one-year-old foliage than for current-year foliage (8 and 6 percent, respectively, for one-year-old and current-year seedling foliage).

The conductance values for the CF treatment exceeded those of the AMB treatment by 2 (one-year-old branch foliage) to 6 (current-year seedling foliage) percent. Mature branch one-year-old foliage was the only tissue type for which there was a substantial difference in percent  $g_s$  decrease for increases in ozone exposure from CF to AMB and from AMB to 2xAMB. In contrast to the other tissue types, increased ozone exposure from CF to AMB levels resulted in a  $g_s$  decrease equal to that resulting from a doubling of ozone from AMB to 2xAMB levels.

#### j. Seasonal variation in ozone effect (O x M)

*One-year-old mature branch foliage* - The seasonal pattern of  $g_s$  by ozone treatment for one-year-old mature branch foliage is shown in Figure 12a. For most months, the greatest  $g_s$  values were observed for the CF treatment while the lowest  $g_s$  values occurred for the 2xAMB treatment. Mean  $g_s$  for the AMB treatment was generally intermediate

and differed very little from that for the CF treatment during the periods of February through April and August through October (Figure 12a). Within months, the maximum deviation in mean  $g_s$  among treatments ranged from less than  $0.001 \text{ mol m}^{-2} \text{ s}^{-1}$  in August to  $0.026 \text{ mol m}^{-2} \text{ s}^{-1}$  in October. Significant differences among treatments were observed for the months of September and October ( $p < 0.05$ , Tukey's HSD). During the last two measurement periods, differences in  $g_s$  between the AMB and 2xAMB treatments were 25 and 34 percent, respectively. The month x ozone effect was statistically significant ( $p = 0.002$ ) for one-year-old mature branch foliage (Table 6).

*Current-year mature branch foliage* - The seasonal variation in ranking of ozone treatment level means for  $g_s$  of current-year branch foliage was very similar to that for one-year-old branch foliage. Generally, the CF treatment had the greatest mean  $g_s$  and the 2xAMB treatment had the least (Figure 12a). Within month maximum differences among ozone treatment means ranged from  $0.004 \text{ mol m}^{-2} \text{ s}^{-1}$  in August to  $0.024 \text{ mol m}^{-2} \text{ s}^{-1}$  in November. Differences between mean  $g_s$  values for the 2xAMB treatment and the CF or AMB treatments increased in October and November. Relative to the AMB treatment, decreases in  $g_s$  for the 2xAMB treatment were 9 and 13 percent, respectively, for the last two measurement dates. In November, the difference between the mean  $g_s$  values for the CF and 2xAMB treatments was significant ( $p < 0.05$ , Tukey's HSD) but the month x ozone interaction effect for the study period was not significant ( $p = 0.157$ , Table 6).

*One-year-old seedling foliage* - With the exceptions of April and May, the greatest  $g_s$  values for one-year-old seedling foliage occurred in the CF treatment (Figure 12b). For all months other than April, May and July, the lowest mean  $g_s$  values were observed for the 2xAMB ozone treatment (Figure 12b). Differences among treatment means were absent from February through May. From June through October, mean values for the CF treatment were greater than those for the 2xAMB treatment ( $p < 0.05$ , Tukey's HSD). Decreases in  $g_s$  at 2xAMB ozone, relative to AMB ozone, in September and October were 30 and 38 percent and significant ( $p < 0.05$ , Tukey's HSD). The consistent ranking of ozone treatment level means among months resulted in a non-significant ( $p = 0.484$ ) month x ozone treatment interaction effect on  $g_s$  for one-year-old seedling foliage (Table 7).

*Current-year seedling foliage* - Mean  $g_s$  of current-year seedling foliage was greatest for the CF treatment for all measurement dates (Figure 12b). Mean  $g_s$  was least for the 2xAMB treatment from August through November (Figure 12b). Mean values for the AMB treatment were intermediate from August through November and were very similar to the CF treatment values from September through November (Figure 12b). Significant differences ( $p < 0.05$ , Tukey's HSD) between CF and 2xAMB mean  $g_s$  values were present in October and November while significant differences ( $p < 0.05$ , Tukey's HSD) between AMB and 2xAMB mean values were present in October. The relative to the AMB treatment, mean  $g_s$  for the 2xAMB treatment was 16 and 12 percent less for October and November, respectively. Over all measurement dates, the month x ozone interaction

effect on  $g_s$  by current-year seedling foliage was not statistically significant ( $p=0.980$ , Table 7).

*Lifestage and foliage age-class comparisons* - For all four combinations of lifestage and age-class, foliage  $g_s$  rates tended to be greatest for tissue in the CF treatment throughout the study and lowest for the 2xAMB treatment from mid-summer to the end of the study (Figures 12a and b). All foliage categories demonstrated an apparent decrease in  $g_s$  values for the 2xAMB treatment relative to the AMB treatment during the latter months of the study period.

The apparent decrease in  $g_s$  due to ozone arose from different seasonal patterns in the  $g_s$  response of foliage to the 2xAMB treatment. Each foliage category had a late-season low in  $g_s$  that occurred in August (one-year-old branch foliage), September (one-year-old and current-year seedling foliage) or October (current-year branch foliage). For all foliage categories, mean  $g_s$  values for the CF and AMB treatments increased from the late-season low through the end of the study period (Figures 12a and b). Mean  $g_s$  values for current-year foliage exposed to 2xAMB also increased following the late-season low, but not to the same extent as did values for the CF and AMB treatments (Figures 12a and b). One-year-old foliage of branches and seedlings exposed to 2xAMB ozone demonstrated either no increase (seedlings) or only a very slight increase (branches) following the late-season low. Thus, the greater apparent decrease in  $g_s$  observed in the late-season arose for reasons that differed between foliage age-classes; a lack of late-season  $g_s$  increase for one-year-old foliage and a relatively small late-season  $g_s$  increase for current-year foliage.

k. Interactive effect of ozone and genotype (O x G)

*One-year-old mature branch foliage* - Mean  $g_s$  of mature branch one-year-old foliage for genotypes 3088 and 3399 tended to be decreased with 2xAMB ozone exposure (Table 13). Genotype 3087  $g_s$  did not vary in response to ozone as treatment level means ranged from 0.061 to 0.064 mol m<sup>-2</sup> s<sup>-1</sup> (Table 13). Among ozone treatments, the ranking of genotype means was varied. Among genotypes, conductance by 3088 ranked highest for the CF treatment and the lowest for the 2xAMB treatment. Genotype 3087 had the lowest mean  $g_s$  value for the CF treatment and the greatest mean  $g_s$  value for the 2xAMB treatment. For the AMB treatment, there was virtually no difference in the mean values for genotypes 3087 and 3399 while the mean value for genotype 3088 was approximately 3 percent less. Decreases in  $g_s$  under 2xAMB ozone, relative to AMB values, for genotypes 3087, 3088 and 3399 were, respectively, 3, 18 and 8 percent. Regardless of the variation in genotype rankings among ozone treatments, the genotype x ozone interaction effect was not significant for one-year-old mature branch foliage ( $p=0.131$ , Table 6).

*Current-year mature branch foliage* - For current-year mature branch foliage, there were consistent declines in  $g_s$  with increasing ozone exposure for all genotypes (Table 13). Decreases in  $g_s$  for 2xAMB relative to AMB ozone were 3, 18 and 8 percent for genotypes 3087, 3088 and 3399, respectively. Mean  $g_s$  values for genotypes 3087 and

3088 were 1 and 9 percent greater, respectively, for the CF treatment relative to the AMB treatment. For genotype 3399, the CF and AMB mean values were equal (Table 13). Conductance decreases for the 2xAMB treatment relative to the AMB treatment ranged from 2 percent for genotypes 3087 and 3088 to 6 percent for genotype 3399. The consistent response to ozone exposure among genotypes was evident by the lack of a significant genotype x ozone interaction term ( $p=0.983$ ) for current-year mature branch foliage (Table 6).

*One-year-old seedling foliage* - There was a significant ( $p=0.015$ ) ozone x genotype interaction effect on  $g_s$  for one-year-old seedling foliage (Table 7). Conductance by genotypes 3088 and 3399 was 3 and 18 percent lower, respectively, in the 2xAMB treatment relative to the AMB treatment (Table 13). Relative to AMB values, the mean  $g_s$  for genotype 3087 was 6 percent greater in the 2xAMB treatment. Thus, in contrast to the other genotypes, there was no apparent ozone reduction for genotype 3087. Mean  $g_s$  values in the CF treatment ranged among genotypes from 0 to 3 percent greater than mean values in the AMB treatment. Among the nine genotype x ozone means, the mean value for genotype 3087 exposed to CF ozone was significantly greater than the mean  $g_s$  value for genotype 3399 exposed to 2xAMB ozone ( $p<0.05$ , Tukeys HSD). No other pair-wise differences among mean values were significant.

*Current-year seedling foliage* - Although the ozone x genotype interaction effect was not significant ( $p=0.491$ , Table 7) for current-year seedling foliage, there was variation among genotypes in the  $g_s$  response to the ozone treatments. Conductance for genotypes 3088 and 3087 tended to decline with increasing ozone concentration (Table 13) as values at 2xAMB ozone were 2 and 15 percent less, respectively, than AMB values for the two genotypes. Relative to AMB values, mean  $g_s$  values for genotypes 3087 and 3088 were, respectively, 4 and 0 percent greater in the CF treatment. In contrast, mean  $g_s$  for genotype 3399 was 8 percent greater in the 2xAMB treatment than in the AMB treatment. Relative to AMB, the mean value for the CF treatment was 15 percent greater for current-year seedling foliage of genotype 3399.

*Lifestage and foliage age-class comparisons* - Response of  $g_s$  to ozone varied among genotypes to a greater extent for seedling foliage than for mature branch foliage. For branches,  $g_s$  tended to decline with increasing ozone exposure regardless of genotype. For seedling foliage, there was at least one genotype that demonstrated a tendency for increased  $g_s$ , relative to AMB values, when exposed to 2xAMB ozone. The lack of an apparent decrease in  $g_s$  at 2xAMB ozone was observed for genotype 3087 one-year-old seedling foliage and for genotype 3399 current-year seedling foliage.

## 1. Chamber effect: Ambient vs Non-chambered Companion

*One-year-old mature branch foliage* - Stomatal conductance by one-year-old mature branch foliage tended to be greater for tissues exposed to AMB conditions than for those exposed to NCAMB conditions (Table 14). Although mean  $g_s$  values for the two treatments differed by 3 percent, they did not differ at the  $p=0.05$  level of significance (Table 14).

*Current-year mature branch foliage* - In contrast to old mature branch foliage, mean  $g_s$  values for current-year branch foliage were 1 percent less for the AMB treatment than those for the NCAMB treatment (Table 14). This slight difference in  $g_s$  among chambered and non-chambered conditions was not significant at the  $p=0.05$  level of significance (Table 14).

*One-year-old seedling foliage* - There was a nearly significant difference ( $p=0.096$ ) in mean  $g_s$  between one-year-old seedling foliage exposed to AMB and NCAMB conditions. Tissues exposed to ambient air in BECs had a mean  $g_s$  value that was 9 percent greater than the mean  $g_s$  value for the non-chambered tissues (Table 14).

*Current-year seedling foliage* - Mean  $g_s$  for current-year seedling foliage was 11 percent lower under AMB conditions than under NCAMB conditions. The difference in  $g_s$  between chambered and non-chambered conditions was not statistically significant ( $p=0.226$ , Table 14).

*Lifestage and foliage age-class comparisons* - The magnitude of the difference in mean  $g_s$  between AMB and NCAMB foliage differed among lifestages. Absolute differences in mean  $g_s$  between the AMB and NCAMB conditions for mature branch foliage were -0.001 for current-year foliage and 0.002 for one-year-old foliage. This suggests that chamber effects were negligible or absent for mature branches. In contrast,  $g_s$  for AMB conditions was  $0.009 \text{ mol m}^{-2} \text{ s}^{-1}$  greater for current-year seedling foliage and  $-0.008 \text{ mol m}^{-2} \text{ s}^{-1}$  less for one-year-old seedling foliage, relative to corresponding means for NCAMB conditions. Regardless of absolute differences, the chamber effect on  $g_s$  was not statistically significant for any of the tissue types.

*Comparison of chamber effect to ozone effect* - Among tissue-types, the decrease in  $g_s$  for 2xAMB ozone, relative to AMB ozone, ranged from 4 to 14 percent. The chamber effect on  $g_s$  ranged from 0 to 10 percent among tissue types. In comparing the ozone and chamber effects, it must be remembered that chamber effects and ozone effects are additive. As defined, the ozone effect is based on a comparison of treatment levels applied to chambered tissues. Thus, for a given lifestage and foliage age-class, all levels of ozone treatment are subject to similar influences induced by a chamber effect and comparisons of ozone response are not confounded.

We must temper our subjective comparisons of ozone effect among tissue types due to the differential effect chamber enclosure may have had on  $g_s$ . The apparent 10

percent reduction in  $g_s$  for one-year-old seedling foliage due to chamber enclosure may have resulted in reduced ozone dose for that tissue type. Similarly the 9 percent increase in  $g_s$  for current-year seedling foliage enclosed in chambers may have effectively increased the ozone dose for that tissue type. The former may have resulted in an underestimate of ozone impact on one-year-old seedling foliage and the latter may have lead to an overestimate of ozone effect.

m. Interactive effect of acidic rain and ozone (A x O)

*One-year-old mature branch foliage* - Regardless of acidic rain treatment,  $g_s$  for one-year-old mature branch foliage tended to decline with increasing ozone exposure (Table 15). The decrease in  $g_s$  for 2xAMB ozone, relative to AMB ozone, was 7, 18 and 14 percent, respectively for the NAP, pH 5.1 and pH 3.0 treatments. Values for the CF treatment exceeded those for the AMB treatment by 4 percent, less than 1 percent, and 3 percent for tissues exposed to NAP, pH 5.1, and pH 3.0 acidic rain treatments, respectively. The acidic rain x ozone interaction effect was not significant for one-year-old mature branch foliage ( $p=0.661$ , Table 6).

*Current-year mature branch foliage* - When exposed to pH 5.1 and pH 3.0 acidic rain,  $g_s$  of current-year mature branch foliage declined with increasing ozone exposure (Table 15). In contrast, similar foliage exposed to NAP did not demonstrate an apparent ozone-related decrease in  $g_s$  as mean values for the AMB and 2xAMB treatments were 0.083 and 0.084 mol m<sup>-2</sup> s<sup>-1</sup>, respectively (Table 15). The percent decrease in  $g_s$  at 2xAMB ozone, relative to AMB ozone, for pH 5.1 and pH 3.0 treatments was 4 and 9 percent, respectively. For all acidic rain levels, the greatest  $g_s$  values were observed for the CF treatment (Table 15). The mean  $g_s$  value for foliage exposed to CF and pH 5.1 was nearly significantly greater ( $p=0.052$ , Tukeys HSD) than the mean values for foliage exposed to 2xAMB and pH 3.0. In spite of the tendency towards different responses to ozone among the various acidic rain treatments, the acidic rain x ozone interaction effect was not statistically significant ( $p=0.726$ , Table 6).

*One-year-old seedling foliage* - There was no significant acidic rain x ozone effect on  $g_s$  for one-year-old seedling foliage ( $p=0.953$ , Table 7). For all acidic rain treatments, mean  $g_s$  declined with increasing ozone exposure. The decrease in  $g_s$  for 2xAMB ozone, relative to AMB ozone, ranged from 3 percent for the pH 3.0 treatment to 11 percent for the NAP treatment. Conductance of tissues exposed to the CF treatment exceeded those for the AMB treatment by less than 1 percent, 2 percent and 14 percent for the NAP, pH 5.1 and pH 3.0 acidic rain treatments, respectively.

*Current-year seedling foliage* - Conductance response to ozone did not differ significantly among acidic rain treatments for current-year seedling foliage ( $p=0.864$ , Table 7). For all acidic rain treatments,  $g_s$  tended to decline with increasing ozone, but the ozone-related decrease in  $g_s$  was negligible (less than 1 percent) for tissues exposed to pH 3.0 acidic rain (Table 15). The decrease in  $g_s$  at 2xAMB ozone, relative to AMB

ozone, was greatest for the NAP treatment (15 percent) and intermediate for the pH 5.1 treatment (3 percent). Values for the CF treatment exceeded those of the AMB treatment by 10, 3 and 6 percent for the NAP, pH 5.1 and pH 3.0 acidic rain treatments, respectively.

*Lifestage and foliage age-class comparisons* - There was a lack of significant acidic rain x ozone interaction effect for all life-stage and foliage age-class combinations suggesting that response to ozone did not vary with acidic rain treatment for any of the foliage types (Tables 6 and 7).

n. Seasonal variation in the interactive effect of acidic rain and genotype  
(M x A x G)

There was a significant ( $p > 0.001$ ) month x acid rain x genotype interaction effect for one-year-old seedling foliage (Table 7). In general, seasonal patterns of conductance were similar for the three genotypes and three acidic rain treatments. Conductance increased to an early season peak, dropped to an early-summer low, increased to a second mid-summer peak and then dropped to a low in late-summer (Figures 13a-c). For seedlings in the NAP and pH 5.1 treatments,  $g_s$  for clone 3087 was generally lower than  $g_s$  for clones 3088 and 3399 (Figures 13a-c). For seedlings subjected to rain of pH 3.0,  $g_s$  values for half-sibs of clone 3087 were slightly greater than or equal to those for clone 3399 and less than those for clone 3088 (Figure 13a-c).

The seasonal patterns also varied among the three rain treatments as  $g_s$  values for the NAP treatment maintained peak spring values through May in contrast to the rain treatments where values declined after April (Figures 13a-c). In the early-spring,  $g_s$  rates tended to be greatest for the pH 3.0 treatment and this trend lasted through June for genotype 3087, through April for genotype 3088 and through March for genotype 3399 (Figures 13a-c). Following a second seasonal peak in July,  $g_s$  values for the pH 3.0 treatment dropped more than did values for the NAP and pH 5.1 treatments. Conductance values were lowest among the acidic rain treatments from July through October for genotype 3087 and from May through October for genotype 3399. For genotype 3088, values for the pH 3.0 did not tend to be lower than those for the NAP and pH 5.1 treatments until October (Figures 13a-c).

o. Seasonal variation in the interactive effect of ozone and genotype (M x O x G)

There was a significant ( $p = 0.043$ ) month x genotype x ozone effect on  $g_s$  for one-year old mature branch foliage (Table 6). The nature of this interaction is difficult to interpret due to the number of factor combinations (3 ozone x 3 genotypes) and measurement dates (9) involved.

Among ozone treatment differences in  $g_s$  were relatively small throughout the season for genotype 3087 (Figure 14a). Apparent differences in  $g_s$  among the AMB and 2xAMB ozone treatments were present in October only (25 percent apparent ozone decrease). Peak  $g_s$  values occurred in March and July for branches in the CF treatment

(Figure 14a). Peak  $g_s$  values for the AMB and 2xAMB treatments were observed in May and July. Seasonal low values occurred in June and August for all ozone treatments (Figure 14a). There were no significant ozone treatment differences, either within or among measurement dates.

Conductance by seedlings of genotype 3088 exposed to AMB and 2xAMB ozone demonstrated relatively large seasonal amplitude (Figure 14b). For branches exposed to 2xAMB, the peak mean value in March was  $0.100 \text{ mol m}^{-2} \text{ s}^{-1}$  and the seasonal low mean value in May was  $0.033 \text{ mol m}^{-2} \text{ s}^{-1}$ , a difference of  $0.067 \text{ mol m}^{-2} \text{ s}^{-1}$ . The mean  $g_s$  for 2xAMB branches in March was substantially greater for genotype 3088 than for the other two genotypes. The difference between maximum (July) and minimum (August) monthly mean values for branches exposed to AMB ozone was also  $0.067 \text{ mol m}^{-2} \text{ s}^{-1}$  (Figure 14b). Branches exposed to CF air had less seasonal amplitude for  $g_s$  ( $0.052 \text{ mol m}^{-2} \text{ s}^{-1}$ ) but did have a distinct seasonal low value in August similar to branches exposed to AMB and 2xAMB ozone. In contrast to genotype 3087, branches of genotype 3088 exposed to CF and AMB ozone demonstrated a substantial late-season increase in  $g_s$  that resulted in means for the AMB treatment being 37 and 45 percent greater than those for the 2xAMB treatment for the months of September and October. In spite of the relatively large ozone-related decreases observed, the differences in  $g_s$  between AMB and 2xAMB mean values were not statistically significant at the  $p=0.05$  level.

Similar to branches of genotype 3087, branches of genotype 3399 demonstrated relatively small seasonal amplitude in  $g_s$  (Figure 14c). Monthly mean  $g_s$  for the CF, AMB and 2xAMB treatments ranged from  $0.053$  to  $0.077 \text{ mol m}^{-2} \text{ s}^{-1}$ , from  $0.049$  to  $0.076 \text{ mol m}^{-2} \text{ s}^{-1}$  and from  $0.041$  to  $0.066 \text{ mol m}^{-2} \text{ s}^{-1}$ , respectively. For genotype 3399, as with genotype 3088,  $g_s$  for the 2xAMB treatment was generally lower than  $g_s$  for CF and AMB treatments over most of the study period (Figures 14b and c). Decreases in conductance at 2xAMB ozone, relative to AMB ozone, for genotype 3399 were 36 and 32 percent for the months of September and October, respectively.

The implications of the significant genotype x ozone x month interaction effect on stomatal conductance by one-year-old foliage of mature branches are likely minor. The source of the interaction was variation in ozone treatment rankings among months. For genotype 3088, there was a large degree of seasonal amplitude in  $g_s$  for the AMB and 2xAMB ozone treatments that was much greater than that observed for the genotypes 3087 and 3399. It should be noted that by the end of the study period,  $g_s$  for mature branches was lower for 2xAMB ozone than for CF or AMB ozone, regardless of genotype. Given the number of mean values included in the interaction effect (81 combinations of genotype, ozone and date), it is highly probable that one or more pair-wise comparisons will be significant, even with relatively imprecise estimates of the means.

Table 6. Summary of mature branch mid-day stomatal conductance repeated measures ANOVA.

Mature Branch Stomatal Conductance RMANOVA								
Source	Current-year Foliage				One-year-old Foliage			
	DF	MS	F	Pr>F	DF	MS	F	Pr>F
Between Subj.								
Acid Rain (A)	2	0.0023	1.92	0.202	2	0.0005	0.36	0.707
Genotype (G)	2	0.0012	1.01	0.402	2	0.0005	0.38	0.694
A x G	4	0.0013	1.05	0.434	4	0.0012	0.84	0.533
Error I	9	0.0012			9	0.0014		
Within Subj.								
Ozone (O)	2	0.0013	2.44	0.115	2	0.0045	10.53	0.001
A x O	4	0.0003	0.51	0.726	4	0.0003	0.61	0.661
G x O	4	0.0001	0.09	0.983	4	0.0009	2.05	0.131
A x G x O	8	0.0004	0.71	0.677	8	0.0007	1.53	0.216
Error II	18	0.0005			18	0.0004		
Month (M)	5	0.0069	5.26	0.001	8	0.0056	7.04	0.001
M x A	10	0.0004	0.28	0.983	16	0.0002	0.27	0.997
M x G	10	0.0009	0.68	0.737	16	0.0014	1.76	0.054
M x A x G	20	0.0008	0.62	0.876	32	0.0007	0.94	0.566
Error III	45	0.0013			72	0.0008		
M x O	10	0.0005	1.49	0.157	16	0.0006	2.56	0.002
M x A x O	20	0.0003	0.81	0.692	32	0.0002	0.99	0.487
M x G x O	20	0.0001	0.43	0.983	32	0.0004	1.55	0.043
M x A x G x O	40	0.0002	0.64	0.941	64	0.0003	1.36	0.069
Error IV	90	0.0003			144	0.0002		

Table 7. Summary of seedling mid-day stomatal conductance repeated measures ANOVA.

Seedling Stomatal Conductance RMANOVA								
Source	Current-year Foliage				One-year-old Foliage			
	DF	MS	F	Pr>F	DF	MS	F	Pr>F
Between Subj.								
Acid Rain (A)	2	0.0220	3.06	0.097	2	0.0046	1.10	0.374
Ozone (O)	2	0.0033	0.46	0.645	2	0.0002	0.05	0.951
A x O	4	0.0022	0.31	0.864	4	0.0008	0.16	0.953
Error I	9	0.0072			9	0.0042		
Within Subj.								
Genotype (G)	2	0.0022	1.72	0.207	2	0.0021	3.77	0.043
A x G	4	0.0009	0.68	0.617	4	0.0007	1.18	0.353
O x G	4	0.0011	0.89	0.491	4	0.0023	4.15	0.015
A x O x G	8	0.0012	0.92	0.522	8	0.0004	0.72	0.673
Error II	18	0.0013			18	0.0006		
Month (M)	5	0.0090	3.59	0.008	7	0.0253	26.64	<0.001
M x A	10	0.0098	3.90	0.001	14	0.0015	1.61	0.101
M x O	10	0.0007	0.29	0.980	14	0.0009	0.98	0.484
M x A x O	20	0.0012	0.48	0.961	28	0.0006	0.61	0.924
Error III	45	0.0025			63	0.0010		
M x G	10	0.0016	1.93	0.051	14	0.0002	0.81	0.657
M x A x G	20	0.0008	0.95	0.526	28	0.0006	2.33	0.001
M x O x G	20	0.0006	0.69	0.828	28	0.0002	0.76	0.793
M x A x O x G	40	0.0007	0.88	0.662	56	0.0003	1.27	0.138
Error IV	90	0.0008			126	0.0003		

Table 8. Stomatal conductance by genotype for one-year-old and current-year foliage of mature branches and seedlings of *Pinus ponderosa*. Values are means and standard errors of the means calculated over all measurement dates, ozone treatments and acid rain treatments. For each lifestage and foliage age-class combination, mean values followed by a common letter do not differ at the  $p=0.05$  probability level.

Stomatal Conductance ( $\text{mol m}^{-2} \text{s}^{-1}$ ) by Genotype					
Lifestage	Foliage Age-class		Genotype		
			3087	3088	3399
Mature Branch	1991	mean	0.063a	0.059a	0.060a
		s.e.	0.005	0.001	0.001
Mature Branch	1992	mean	0.081a	0.087a	0.084a
		s.e.	0.002	0.004	0.004
Seedling	1991	mean	0.063b	0.069a	0.065ab
		s.e.	0.002	0.002	0.002
Seedling	1992	mean	0.108a	0.114a	0.117a
		s.e.	0.003	0.003	0.005

Table 9. Stomatal conductance by acid rain treatment for one-year-old and current-year foliage of mature branches and seedlings of *Pinus ponderosa*. Acid rain treatments include no acid rain (NAP), pH 5.1 simulated rain (pH 5.1) and pH 3.0 simulated rain (pH 3.0). Values are means and standard errors of the means calculated over all measurement dates, ozone treatments and genotypes. For each lifestage and foliage age-class combination, mean values followed by a common letter do not differ at the p=0.05 probability level.

Stomatal Conductance (mol m <sup>-2</sup> s <sup>-1</sup> ) by Acid Rain Treatment					
Lifestage	Foliage Age-class		Acid Rain Treatment		
			NAP	pH 5.1	pH 3.0
Mature Branch	1991	mean	0.059a	0.061a	0.062a
		s.e.	0.002	0.001	0.004
Mature Branch	1992	mean	0.084a	0.088a	0.079a
		s.e.	0.004	0.002	0.004
Seedling	1991	mean	0.066a	0.065a	0.067a
		s.e.	0.003	0.003	0.002
Seedling	1992	mean	0.110a	0.121a	0.108a
		s.e.	0.005	0.006	0.004

Table 10. Stomatal conductance by acid rain treatment and genotype for one-year-old and current-year foliage of mature branch and seedling foliage of *Pinus ponderosa*. Acid rain treatments include no acid rain (NAP), pH 5.1 simulated rain (pH 5.1) and pH 3.0 simulated rain (pH 3.0). Genotypes include 3087, 3088 and 3399. Values are means and standard errors of the means calculated over all measurement dates and ozone treatments. For each life stage and foliage age-class combination, mean values followed by a common letter do not differ at the  $p=0.05$  probability level.

Stomatal Conductance (mol m <sup>-2</sup> s <sup>-1</sup> ) by Acid Rain Treatment and Genotype						
Parameter	NAP		pH 5.1		pH 3.0	
	3087	3088	3399	3087	3088	3399
Mature Branch One-year-old Foliage						
mean	0.055a	0.060a	0.061a	0.063a	0.060a	0.058a
s.e.	0.007	0.004	0.002	0.002	0.001	0.000
Mature Branch Current-year Foliage						
mean	0.076a	0.088a	0.088a	0.083a	0.092a	0.091a
s.e.	0.000	0.011	0.002	0.000	0.006	0.001
Seedling One-year-old Foliage						
mean	0.064a	0.069a	0.065a	0.061a	0.067a	0.066a
s.e.	0.004	0.003	0.004	0.004	0.003	0.004
Seedling Current-year Foliage						
mean	0.107b	0.112ab	0.111ab	0.112ab	0.123ab	0.129a
s.e.	0.006	0.005	0.007	0.004	0.006	0.009
				0.106b	0.108b	0.111b
				0.006	0.005	0.007

Table 11. Stomatal conductance for one-year-old and current-year foliage of mature branch and seedling foliage of *Pinus ponderosa* under ambient ozone and exposed to either no acid rain (NAP) or pH 5.1 rain (pH 5.1). Values are means and standard errors of the means calculated over all measurement dates and genotypes. For each lifestage and foliage age-class combination, probability values indicate the likelihood that differences between means do not differ significantly at the  $p=0.05$  level.

Effect of Acid Rain Application on Stomatal Conductance				
Parameter	Stomatal Conductance ( $\text{mol m}^{-2} \text{s}^{-1}$ )		t-test	
	NAP	pH 5.1	t value	P<t
Mature Branch One-year-old Foliage				
mean	0.059	0.065	1.372	0.200
s.e.	0.003	0.002		
Mature Branch Current-year Foliage				
mean	0.083	0.088	0.701	0.499
s.e.	0.005	0.004		
Seedling One-year-old Foliage				
mean	0.068	0.067	0.169	0.871
s.e.	0.007	0.003		
Seedling Current-year Foliage				
mean	0.112	0.121	0.831	0.438
s.e.	0.009	0.006		

Table 12. Stomatal conductance by ozone treatment for one-year-old and current-year foliage of mature branches and seedlings of *Pinus ponderosa*. Ozone treatments include charcoal filtered ambient air (CF), ambient air (AMB) and air supplemented with ozone to twice ambient ozone concentration (2xAMB). Values are means and standard errors of the means calculated over all measurement dates, acidic rain treatments and genotypes. For each lifestage and foliage age-class combination, mean values followed by a common letter do not differ at the  $p=0.05$  probability level.

Stomatal Conductance ( $\text{mol m}^{-2} \text{s}^{-1}$ ) by Ozone Treatment					
Lifestage	Foliage Age-class		Ozone Treatment		
			CF	AMB	2xAMB
Mature Branch	1991	mean	0.064a	0.063a	0.054b
		s.e.	0.002	0.002	0.002
Mature Branch	1992	mean	0.088a	0.084a	0.081a
		s.e.	0.003	0.003	0.003
Seedling	1991	mean	0.070a	0.066a	0.061a
		s.e.	0.003	0.004	0.002
Seedling	1992	mean	0.120a	0.113a	0.106a
		s.e.	0.006	0.005	0.006

Table 13. Stomatal conductance by ozone treatment and genotype for one-year-old and current-year foliage of mature branch and seedling foliage of *Pinus ponderosa*. Ozone treatments include charcoal-filtered (CF), ambient (AMB) and twice ambient (2xAMB) atmospheric ozone concentration. Genotypes include 3087, 3088 and 3399. Values are means and standard errors of the means calculated over all measurement dates and ozone treatments. For each lifestage and foliage age-class combination, mean values followed by a common letter do not differ at the  $p=0.05$  probability level.

Stomatal Conductance (mol m <sup>-2</sup> s <sup>-1</sup> ) by Ozone Treatment and Genotype								
CF			AMB			2xAMB		
Parameter	3087	3088	3399	3087	3088	3399	3087	3399
Mature Branch One-year-old Foliage								
mean	0.063a	0.067a	0.063a	0.064a	0.061a	0.063a	0.061a	0.052a
s.e.	0.005	0.003	0.004	0.005	0.003	0.002	0.005	0.002
Mature Branch Current-year Foliage								
mean	0.084a	0.092a	0.087a	0.080a	0.086a	0.085a	0.078a	0.080a
s.e.	0.002	0.006	0.006	0.003	0.005	0.005	0.005	0.004
Seedling One-year-old Foliage								
mean	0.067ab	0.073a	0.070ab	0.059ab	0.072ab	0.068ab	0.063ab	0.057b
s.e.	0.005	0.003	0.004	0.004	0.003	0.004	0.003	0.003
Seedling Current-year Foliage								
mean	0.112a	0.120a	0.128a	0.108a	0.120a	0.111a	0.105a	0.112a
s.e.	0.005	0.004	0.010	0.005	0.005	0.006	0.006	0.007

Table 14. Stomatal conductance for one-year-old and current-year foliage of mature branch and seedling foliage of *Pinus ponderosa* exposed to ambient ozone either in BECs (AMB) or as non-chambered companion tissues (NCAMB). Values are means and standard errors of the means calculated over all acid rain treatments, genotypes and measurement dates. For each lifestage and foliage age-class combination, probability values indicate the likelihood that differences between mean do not differ significantly at the  $p=0.05$  level.

Chamber Effect on Stomatal Conductance: Ambient versus Non-chambered Companions						
Lifestage	Foliage Age-class	Parameter	Stomatal Conductance (mol m <sup>-2</sup> s <sup>-1</sup> )		t-test	
			AMB	NCAMB	t value	P>t
Branch	1991	mean	0.063a	0.061a	0.664	0.511
		s.e.	0.002	0.002		
Branch	1992	mean	0.084a	0.085a	0.260	0.796
		s.e.	0.003	0.003		
Seedling	1991	mean	0.066a	0.074a	1.770	0.096
		s.e.	0.003	0.003		
Seedling	1992	mean	0.113a	0.104a	1.260	0.226
		s.e.	0.005	0.005		

Table 15. Stomatal conductance by acid rain treatment and ozone treatment for one-year-old and current-year foliage of mature branch and seedling foliage of *Pinus ponderosa*. Acid rain treatments include no acid rain (NAP), pH 5.1 simulated rain (pH 5.1) and pH 3.0 simulated rain (pH 3.0). Ozone treatments include charcoal filtered (CF), ambient (AMB), and twice ambient (2xAMB) ozone concentration. Values are means and standard errors of the means calculated over all measurement dates and ozone treatments. For each life stage and foliage age-class combination, mean values followed by a common letter do not differ at the  $p=0.05$  probability level.

Stomatal Conductance (mol m <sup>-2</sup> s <sup>-1</sup> ) by Acid Rain Treatment and Ozone Treatment									
NAP			pH 5.1			pH 3.0			
Parameter	CF	AMB	2xAMB	CF	AMB	2xAMB	CF	AMB	2xAMB
Mature Branch One-year-old Foliage									
mean	0.062a	0.060a	0.055a	0.065a	0.065a	0.052a	0.067a	0.065a	0.054a
s.e.	0.004	0.003	0.002	0.005	0.002	0.004	0.003	0.004	0.006
Mature Branch Current-year Foliage									
mean	0.085a	0.083a	0.084a	0.094a	0.088a	0.084a	0.084a	0.080a	0.073a
s.e.	0.005	0.005	0.004	0.005	0.004	0.003	0.005	0.005	0.005
Seedling One-year-old Foliage									
mean	0.068a	0.068a	0.061a	0.068a	0.067a	0.060a	0.073a	0.064a	0.062a
s.e.	0.002	0.007	0.003	0.008	0.003	0.004	0.001	0.005	0.003
Seedling Current-year Foliage									
mean	0.123a	0.112a	0.095a	0.125a	0.121a	0.118a	0.112a	0.106a	0.106a
s.e.	0.004	0.009	0.009	0.015	0.006	0.009	0.007	0.008	0.007

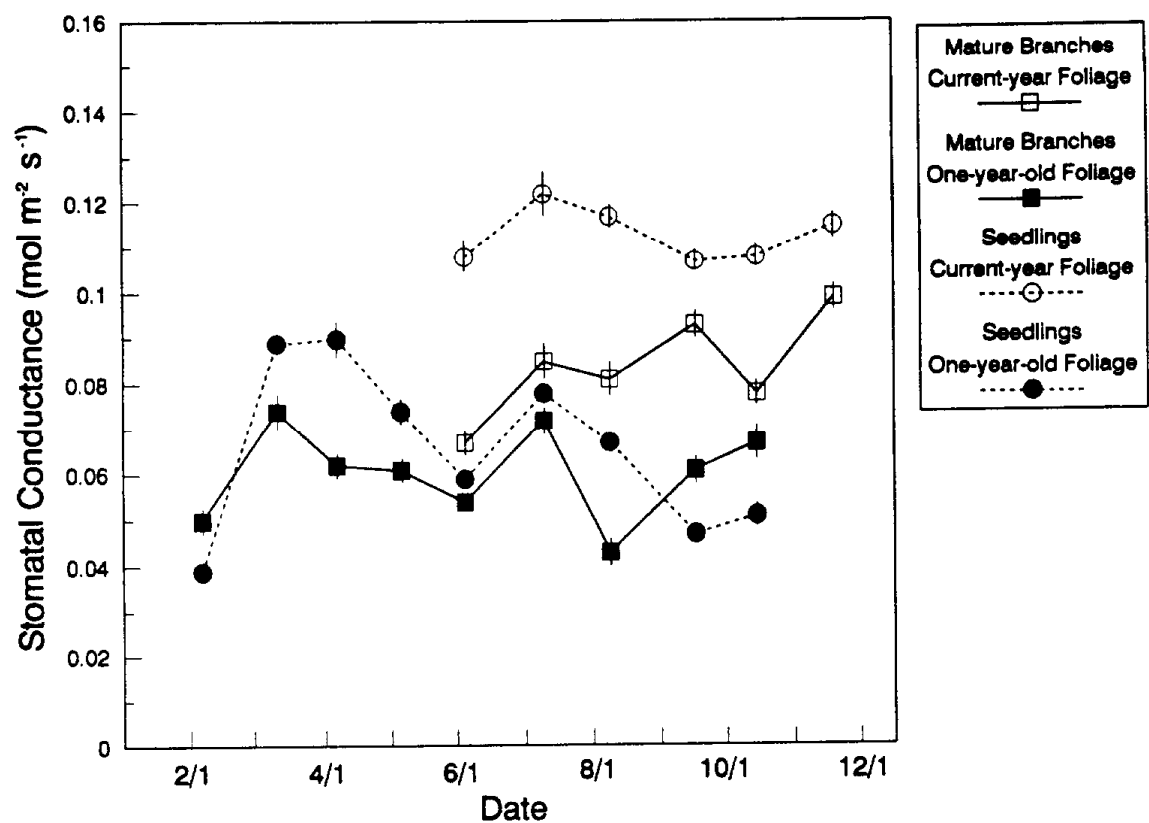


Figure 8. Seasonal variation in mid-day stomatal conductance for current-year (1992) and one-year-old (1991) foliage of *Pinus ponderosa* a) mature branches and b) seedlings. Values are means calculated over all genotypes and pollutant treatments. Vertical lines represent one standard error of the mean.

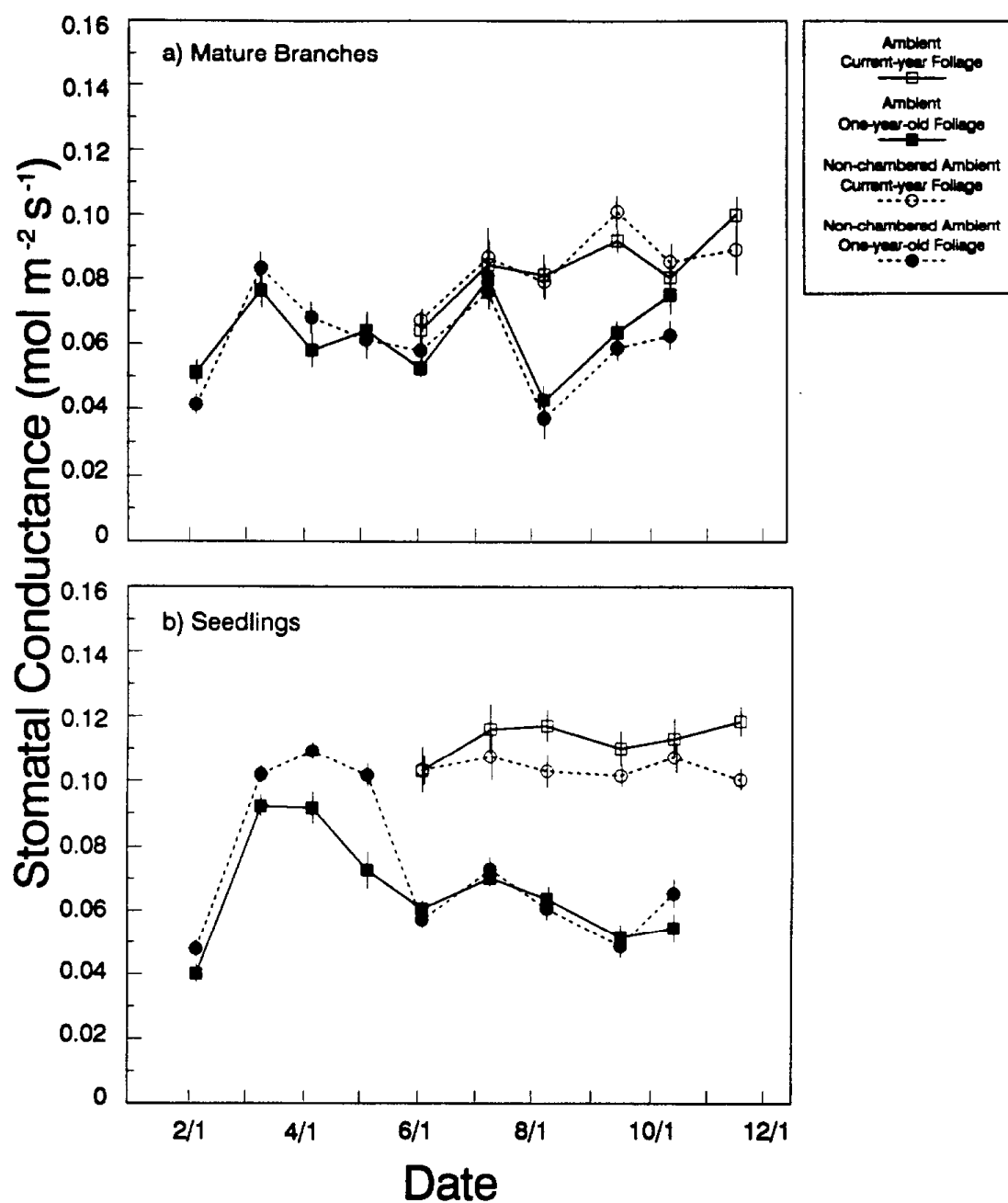


Figure 9. Seasonal variation in mid-day stomatal conductance for current-year and one-year-old foliage of *Pinus ponderosa* a) mature branches and b) seedlings exposed to ambient ozone in BECs (AMB) or non-chambered ambient ozone (NCAMB). Values are means over all genotypes and acidic rain treatments. Vertical lines represent one standard error of the mean.

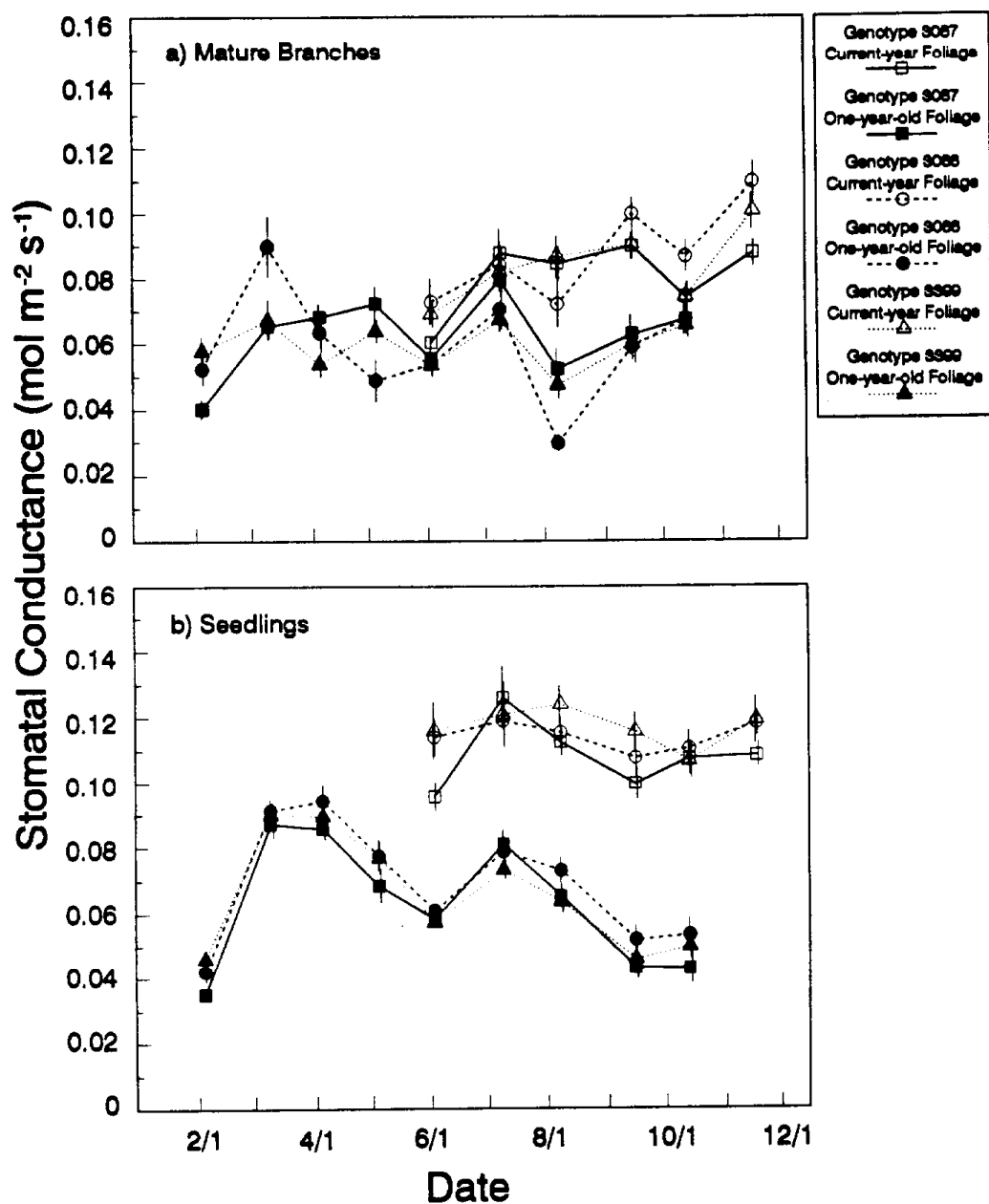


Figure 10. Seasonal variation in mid-day stomatal conductance for current-year and one-year-old foliage of *Pinus ponderosa* a) mature branches and b) seedlings of genotypes 3087, 3088 and 3399. Values are means over all acidic rain and ozone treatments. Vertical lines represent one standard error of the mean.

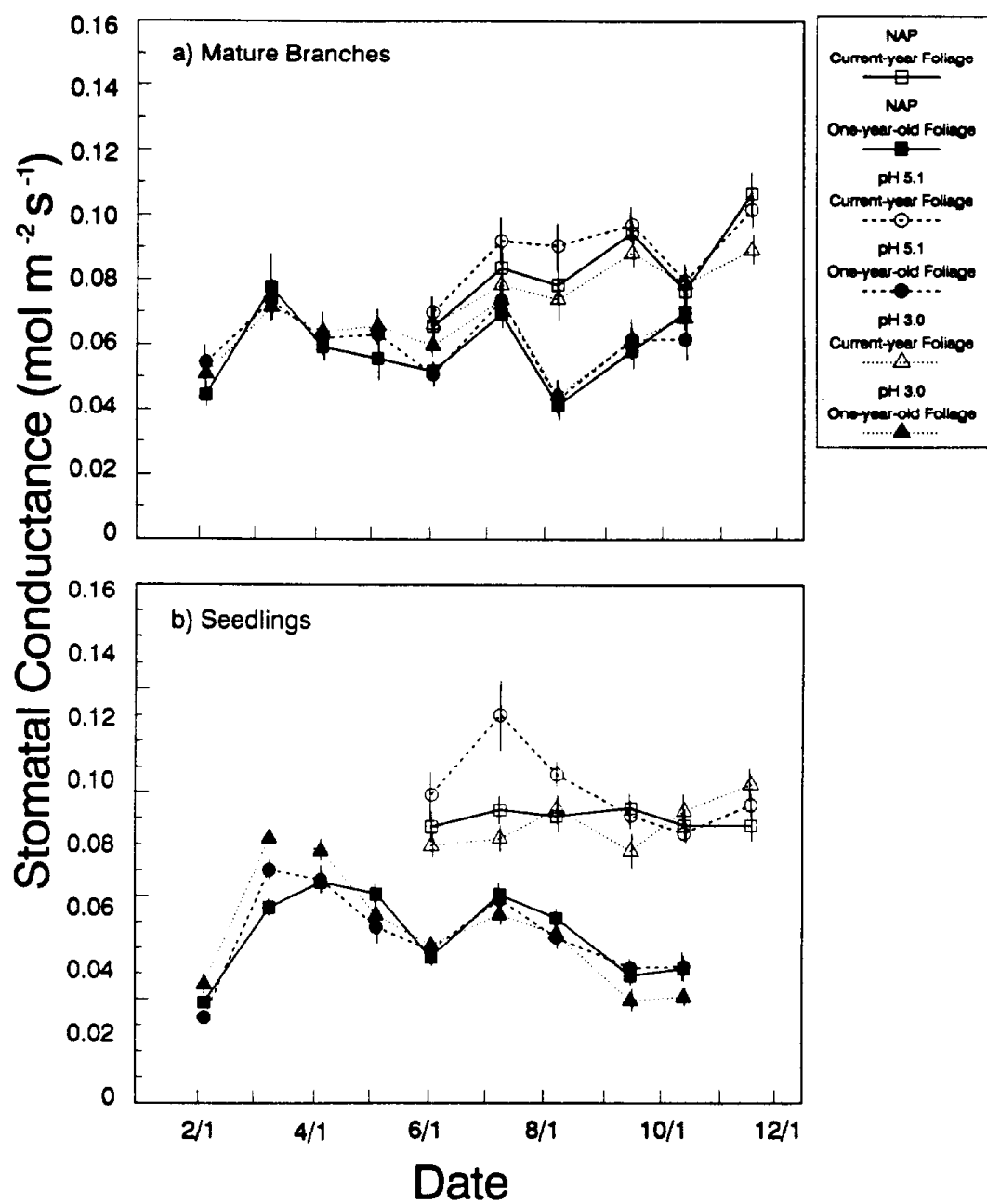


Figure 11. Seasonal variation in mid-day stomatal conductance for current-year and one-year-old foliage of *Pinus ponderosa* a) mature branches and b) seedlings exposed to no acid rain (NAP), pH 5.1 rain (pH 5.1) or pH 3.0 rain (pH 3.0). Values are means over all genotypes and ozone treatments. Vertical lines represent one standard error of the mean.

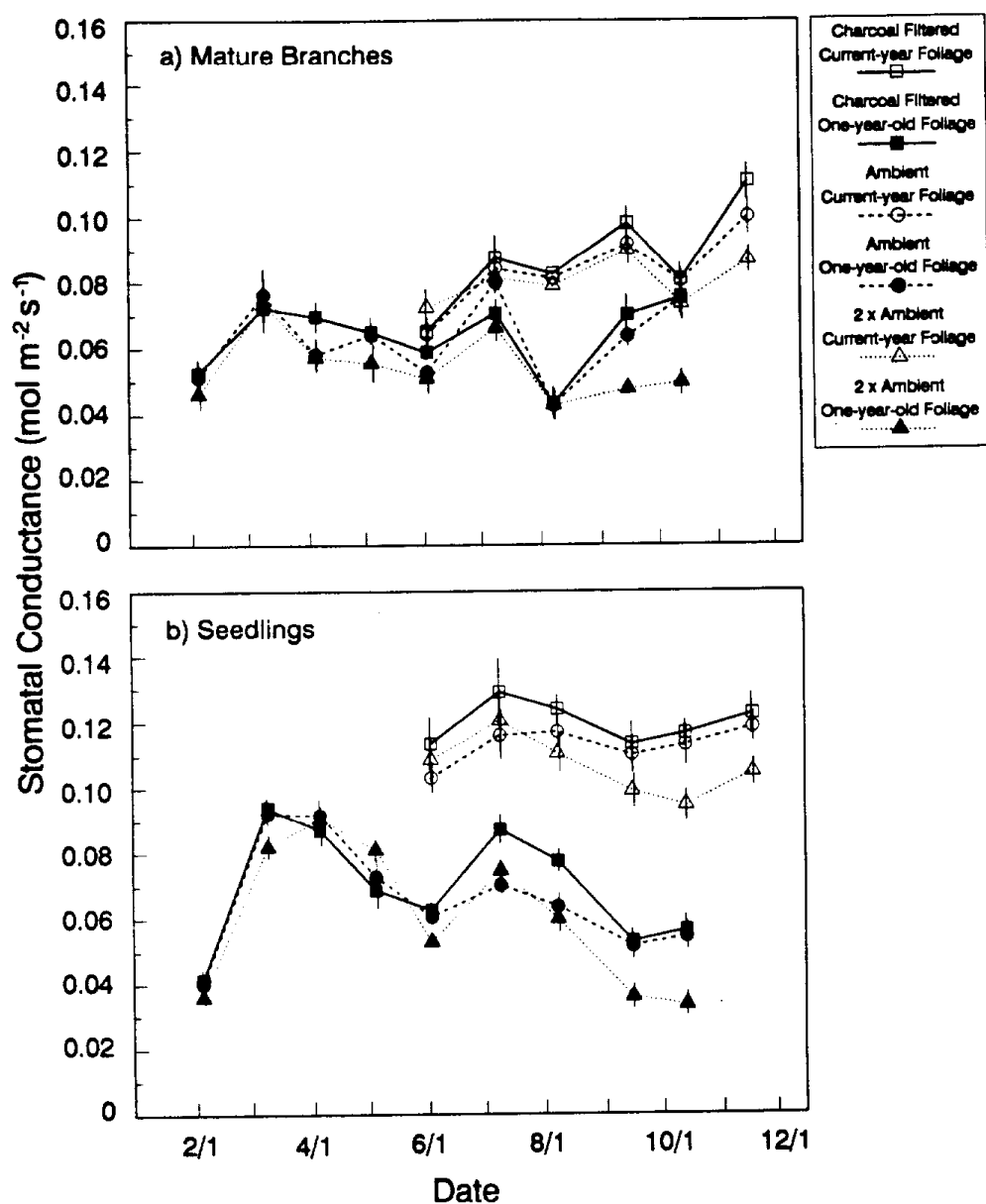


Figure 12. Seasonal variation in mid-day stomatal conductance for current-year and one-year-old foliage of *Pinus ponderosa* a) mature branches and b) seedlings exposed to charcoal-filtered air (CF), ambient ozone (AMB) or twice ambient ozone (2xAMB) in BECs. Values are means over all genotypes and ozone treatments. Vertical lines represent one standard error of the mean.

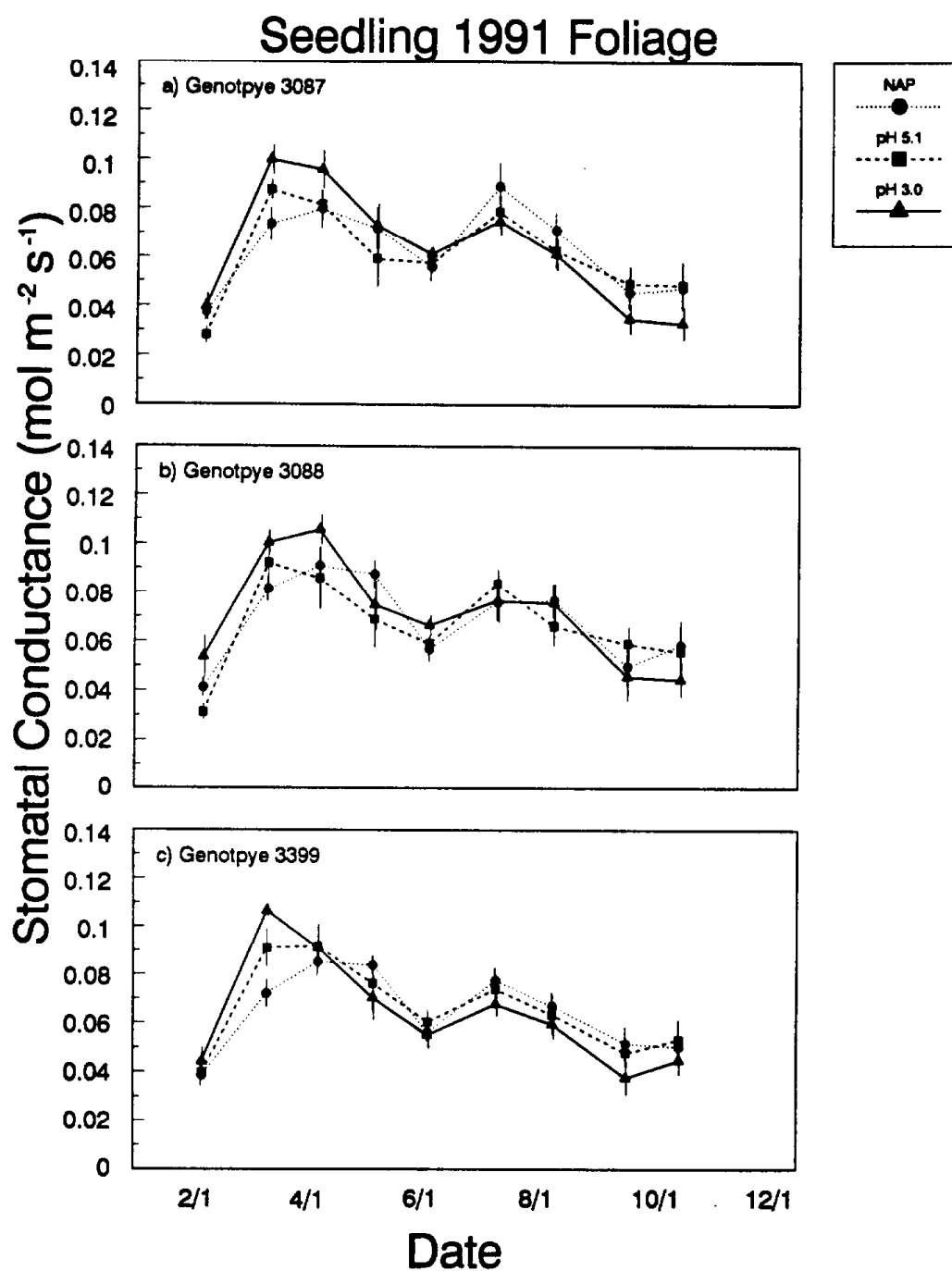


Figure 13. Seasonal variation in mid-day stomatal conductance for one-year-old foliage *Pinus ponderosa* half-sib seedlings genotypes exposed to no acid rain (NAP), pH 5.1 rain or pH 3.0 rain. Values are means over all ozone treatments for genotypes a) 3087, b) 3088, and c) 3399. Vertical lines represent one standard error of the mean.

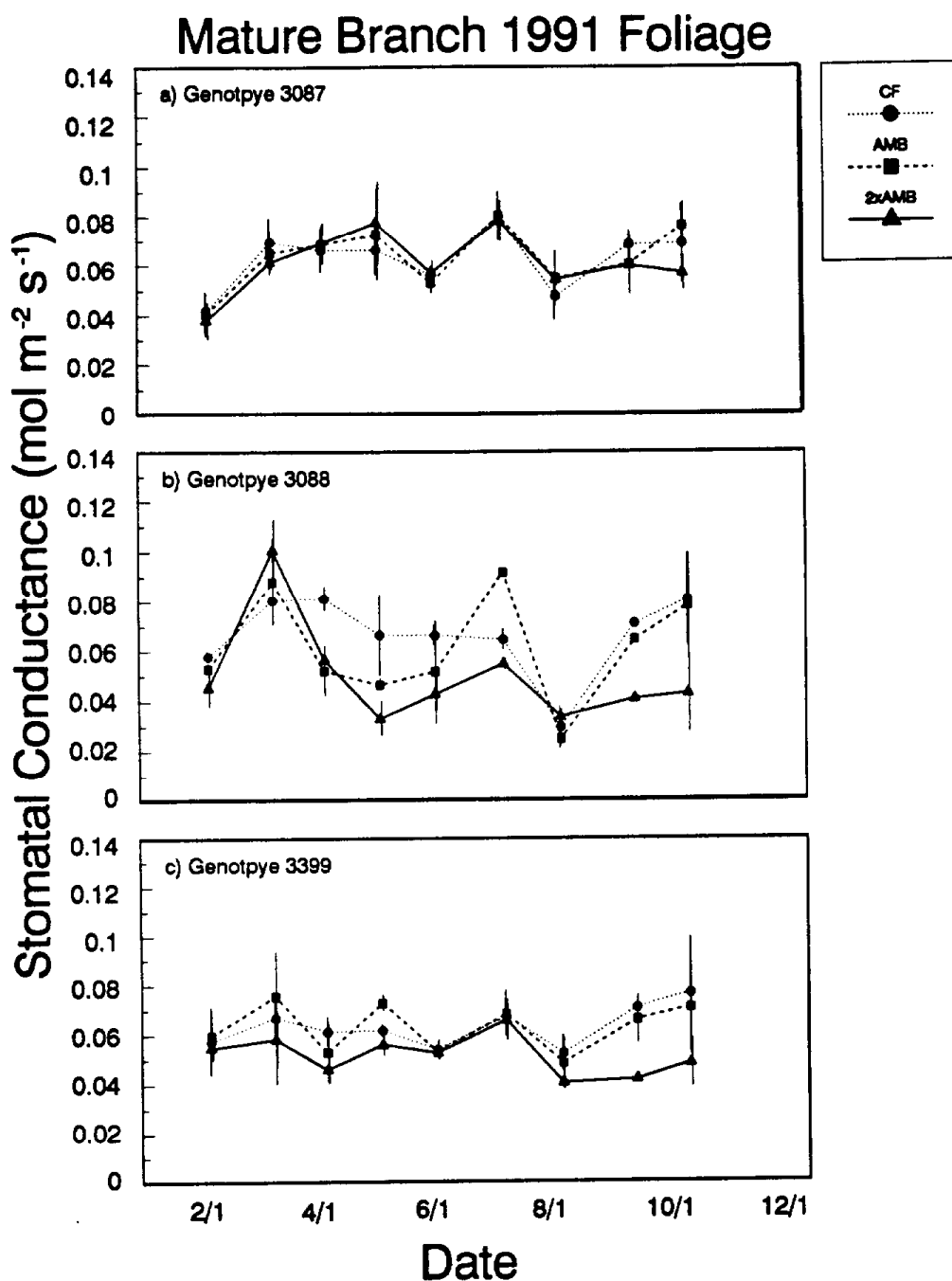


Figure 14. Seasonal variation in mid-day stomatal conductance for one-year-old foliage *Pinus ponderosa* mature branches exposed to charcoal filtered air (CF), ambient ozone (AMB) or twice ambient ozone (2xAMB). Values are means over all acidic rain treatments for genotypes a) 3087, b) 3088, and c) 3399. Vertical lines represent one standard error of the mean.

## 2. Net Photosynthesis

### a. Seasonal pattern of net photosynthesis over all genotypes and pollutant treatments (M)

*One-year-old mature branch foliage* - The seasonal pattern of net photosynthesis ( $P_n$ ) for one-year-old foliage of mature branches is presented in Figure 15. Monthly mean  $P_n$  ranged from a low of  $2.30 \mu\text{mol m}^{-2} \text{s}^{-1}$  in August to a high of  $4.95 \mu\text{mol m}^{-2} \text{s}^{-1}$  in March. During the season three peak values were observed in the months of March, July, and October (Figure 15). Low values were observed in February, June and August. The significant increase ( $p < 0.05$ , Tukey's HSD) in  $P_n$  from June to July coincided with the 4-week period in which ozone fumigation was interrupted. The increase in  $P_n$  from August to October was approximately  $1.93 \mu\text{mol m}^{-2} \text{s}^{-1}$  and the mean for October was 85 percent of the maximum value observed in March.

*Current-year mature branch foliage* - Net photosynthesis of current-year mature branch foliage increased over the study period from  $2.90 \mu\text{mol m}^{-2} \text{s}^{-1}$  in June to  $5.38 \mu\text{mol m}^{-2} \text{s}^{-1}$  in November (Figure 15). There was a very highly significant effect of measurement date on  $P_n$  ( $p < 0.001$ , Table 16). Mean values for September through November were significantly greater ( $p < 0.05$ , Tukey's HSD) than those for June through August. Mean  $P_n$  for June was significantly less ( $p < 0.05$ , Tukey's HSD) than the mean  $P_n$  for July and August.

*One-year-old seedling foliage* - The seasonal  $P_n$  pattern for one-year-old seedling foliage was similar to that for one-year-old branch foliage (Figure 15). There was a significant effect of measurement date on  $P_n$  ( $p < 0.001$ , Table 17) as the minimum and maximum monthly mean values were  $2.05$  and  $5.56 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively. Maximum  $P_n$  values were observed in March and a second, lesser, peak was observed in July. Low values occurred in February, June and September. The late-season  $P_n$  increase was not as substantial as that observed for one-year-old branch foliage as the mean value for October was only 26 percent greater than the value for September and only 53 percent as great as the maximum value observed in March.

*Current-year seedling foliage* - Mean  $P_n$  for current-year seedling foliage increased significantly from June to August ( $p < 0.05$ , Tukey's HSD). With the exception of an increase in October, there were no differences in  $P_n$  among dates for August through November (Figure 15). The monthly mean values ranged from  $3.63$  to  $5.78 \mu\text{mol m}^{-2} \text{s}^{-1}$  and the effect of measurement date was highly significant ( $p < 0.001$ , Table 17).

*Lifestage and foliage age-class comparisons* - Within foliage age-classes, there was little difference in the seasonal pattern of mid-day  $P_n$  for mature branches and seedlings. For one-year-old foliage, a bimodal pattern with peak values occurring in March and July was observed for foliage of both life-stages. The late season low occurred in August for branches and a month later, in September, for seedlings. Perhaps the biggest difference

between mature branches and seedlings was the magnitude of the late-season increase in  $P_n$ , which tended to be much less for seedlings. As a result, the rates of  $P_n$  observed in October and September were, respectively, 56 and 63 percent greater for mature branches than for seedlings.

Photosynthetic rates for current-year foliage of both branches and seedlings increased substantially from early- to late-summer. The late summer plateau began in August for seedlings and in September for mature branches. Photosynthetic rates for current-year foliage tended to be greater than those of branches throughout much of the season although differences between life-stages diminished late in the study.

b. Chamber effect on the seasonal pattern of net photosynthesis

To assess effect of chamber enclosure on  $P_n$ , monthly mean values for the ambient ozone BECs (AMB) and non-chambered companions (NCAMB) are compared. The mean values are calculated over all genotypes and acid rain treatment levels.

*One-year-old mature branch foliage* - Over the study, there was little difference in the seasonal pattern of  $P_n$  between AMB and NCAMB treatments for one-year-old foliage of mature branches. For both treatments, the seasonal pattern was very similar to that described for the tissue class in general in section 2a (Figures 15 and 16). Net photosynthesis tended to be greater for AMB than for NCAMB in all months other than March and April. Absolute differences in monthly values for the two treatments ranged from  $0.014 \mu\text{mol m}^{-2} \text{s}^{-1}$  in June to  $1.10 \mu\text{mol m}^{-2} \text{s}^{-1}$  in October. Differences between AMB and NCAMB mean  $P_n$  values were significant only in February ( $p=0.021$ , t-test) and October ( $p=0.004$ , t-test).

*Current-year mature branch foliage* - Significant differences in  $P_n$  between AMB and NCAMB treatments were absent for current-year mature branch foliage. The seasonal patterns of  $P_n$  for the AMB and NCAMB treatments were very similar to those described for current-year branch foliage in section 2a (Figures 15 and 16).

*One-year-old seedling foliage* - Throughout the study period, mean  $P_n$  for one-year-old seedling foliage tended to be greater for NCAMB seedlings than for AMB seedlings (Figure 16b). Differences in  $P_n$  between the treatments, which ranged from  $0.04$  to  $1.40 \mu\text{mol m}^{-2} \text{s}^{-1}$ , were greatest from March through May. Mean  $P_n$  was significantly greater for the NCAMB treatment in April ( $p=0.005$ ) and May ( $p<0.001$ ). In general, the seasonal patterns for the AMB and NCAMB treatments were very similar to that described for the tissue class as a whole in section 2a.

*Current-year seedling foliage* - For the months of August through November, mean  $P_n$  of current-year foliage tended to be greater for AMB seedlings than for NCAMB seedlings (Figure 16b). Differences in  $P_n$  between the two treatments ranged from  $0.21 \mu\text{mol m}^{-2} \text{s}^{-1}$  in July to  $0.88 \mu\text{mol m}^{-2} \text{s}^{-1}$  in August. Non-chambered seedlings did not have the relatively large increase in  $P_n$  from July to August that was present for AMB

seedlings (Figure 16b) or for current-year seedling foliage as a whole (Figure 15). As a result, the difference in mean  $P_n$  between AMB and NCAMB seedlings was significant ( $p=0.008$ ) for the month of August.

*Lifestage and foliage age-class comparisons* - The seasonal pattern of  $P_n$  was not substantially different between AMB and NCAMB conditions for one-year-old and current-year foliage of mature branches. In contrast,  $P_n$  for one-year-old seedling foliage was negatively impacted by chamber enclosure during a two-to-three month period in the spring. With the exception of one-year-old seedling foliage, there was a weak trend for  $P_n$  values to be greater under AMB conditions. In general, tissues subjected to both AMB and NCAMB condition demonstrated seasonal  $P_n$  patterns similar to those described in section 2a.

### c. Genotype effect (G)

*One-year-old mature branch foliage* - There was significant variation in  $P_n$  among genotypes for one-year-old mature branch foliage ( $p=0.015$ , Table 16). When averaged over all measurement dates, ozone treatments and acidic rain treatments, mean values for genotypes 3087, 3088 and 3399 were, respectively, 4.00, 3.42 and 3.80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Table 18). Mean  $P_n$  for genotype 3087 was greater than that for genotype 3088. The mean value for genotype 3399 did not differ from that for either genotype 3087 or genotype 3088.

*Current-year mature branch foliage* - Genotype 3399 had the greatest mean  $P_n$  and genotype 3088 had the lowest mean  $P_n$  among families for current-year mature branch foliage (Table 18). The mean value for genotype 3399 was significantly greater than that for genotype 3088 ( $p<0.05$ , Tukey's HSD). The differences in mean values between genotype 3087 and either genotypes 3088 or 3399 were not statistically significant.

*One-year-old seedling foliage* - For one-year-old seedling foliage, there was no significant genotype effect on  $P_n$  ( $p=0.547$ , Table 17). Mean  $P_n$  ranged from 3.33  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for genotype 3399 to 3.42  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for genotype 3088 (Table 18).

*Current-year seedling foliage* - The genotype effect was not statistically significant ( $p=0.130$ , Table 17) for current-year seedling foliage. Mean values were 4.67, 4.94 and 4.87 for genotypes 3087, 3088 and 3399, respectively.

*Lifestage and foliage age-class comparisons* - Genotype had more effect on  $P_n$  for mature branch foliage than for seedling foliage, regardless of age-class. For mature branches,  $P_n$  for genotype 3399 was greater than  $P_n$  for genotype 3088. Although differences among genotypes were not significant for seedlings, genotype 3088 displayed the greatest mean values for both foliage age-classes. Thus, among life-stages there was a distinct difference in the relative performance of the genotypes. It is also important to note that the seedlings were actually half-sib representations of the genotypes while the

mature branches are clonal representations. As a result, the comparison between life-stages may be confounded by the variation in paternal contribution to the half-sib seedling genomes.

d. Seasonal variation in genotype effect (G x M)

*One-year-old mature branch foliage* - There was no significant genotype x month interaction for one-year-old mature branch foliage ( $p=0.135$ , Table 16). With the exceptions of February and September, mean  $P_n$  was greatest for genotype 3087 (Figure 17a). From May through October, genotype 3088 had the lowest mean  $P_n$ . The relative ranking for genotype 3399 varied widely from February through March but from April through October,  $P_n$  for genotype 3399 was either slightly less than or equal to that for genotype 3087. Significant differences among genotypes were observed only in August when  $P_n$  for genotype 3088 was significantly less than that for genotype 3087 ( $p=0.032$ , Tukey's HSD). Although all three genotypes demonstrated lower  $P_n$  in August, the decrease relative to July rates was substantially greater for genotype 3088 than for the other genotypes (Figure 17a).

*Current-year mature branch foliage* - With the exception of September and October,  $P_n$  for current-year branch foliage was greatest for genotype 3399. Mean Values for genotype 3088 were the lowest among genotypes from June through August. Genotype 3088 displayed a large reduction in  $P_n$  during August that was not present for either genotypes 3087 or 3399 (Figure 17a). As a result, there was a significant difference among genotype means in August ( $p=0.017$ , Tukey's HSD) as the value for genotype 3088 was significantly less than that of genotype 3399. From September through November, differences among genotype means were not substantial. Even with the differences among mean in August, there was not a significant genotype x month interaction effect for current-year branch foliage ( $p=0.190$ , Table 16).

*One-year-old seedling foliage* - Within measurement periods, there was little difference in mean  $P_n$  values among genotypes for one-year-old seedling foliage. The difference between minimum and maximum genotype mean values for a given month ranged from  $0.07 \mu\text{mol m}^{-2} \text{s}^{-1}$  in March to  $0.65 \mu\text{mol m}^{-2} \text{s}^{-1}$  in July (Figure 17b). Differences among genotypes were not significant at the  $p=0.05$  level for any month. Over the study period, the genotype x month interaction effect on  $P_n$  was not significant ( $p=0.68$ , Table 17).

*Current-year seedling foliage* - There was little difference in  $P_n$  among genotypes within measurement periods for current-year seedling foliage but there was substantial variation among months in the relative ranking of genotype means (Figure 17b). Among genotypes, 3088 had the greatest  $P_n$  rates in the months of June, July, October and November. Genotype 3399 had the greatest mean values in the months of August and September. All three genotypes had the lowest ranking at some point during the study. Differences among genotypes were not significant ( $p=0.05$ ) for any measurement period.

In spite of the lack of within-month differences, the variation in relative genotype rankings resulted in a significant genotype x month interaction effect of  $P_n$  ( $p=0.021$ , Table 17).

*Lifestage and foliage age-class comparisons* - There was a lack of significant genotype x month interaction effect for mature branches and for one-year-old foliage of seedlings. The significant interaction effect for current-year seedling foliage was not the result of substantial differences in  $P_n$  among genotypes, but was due to the large monthly variation in the relative ranking of genotype mean values. The variation in ranking was large, but the differences in genotype performance were small. Thus, for all categories of foliage, response to seasonal changes in environment did not result in substantial changes in the relative  $P_n$  performance of the three genotypes.

e. Acidic rain effect (A)

*One-year-old mature branch foliage* - Treatment with acidic rain had no significant effect on  $P_n$  for one-year-old mature branch foliage ( $p=0.174$ , Table 16). When averaged over all measurement dates, genotypes and ozone treatments, mean values were 3.55, 3.81 and 3.86  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for the NAP, pH 5.1 and pH 3.0 treatments, respectively (Table 19). It should be noted that there was a slight, although non-significant, increase in  $P_n$  with exposure to both pH 5.1 or pH 3.0 rain.

*Current-year mature branch foliage* - Current-year mature branch foliage demonstrated a trend for decreasing  $P_n$  with increasing acidity exposure. Mean values for the NAP, pH 5.1 and pH 3.0 treatments were 4.55, 4.45 and 4.36  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively (Table 19). Differences in  $P_n$  were not significant ( $p=0.526$ , Table 16).

*One-year-old seedling foliage* - The effect of acidic rain on  $P_n$  was not significant for one-year-old seedling foliage ( $p=0.880$ , Table 17). Mean values ranged from 3.30  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for pH 5.1 to 3.46  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for NAP (Table 19).

*Current-year seedling foliage* - Acidic rain effects on  $P_n$  were absent for current-year seedling foliage ( $p=0.126$ , Table 17). Mean values for the NAP, pH 5.1 and pH 3.0 treatments were 4.96, 5.04 and 4.49  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively (Table 19).

*Lifestage and foliage age-class comparisons* - Of the four combinations of lifestage and foliage age-class, there were no significant main effects of acidic rain on  $P_n$ . The relative ranking of acidic rain treatment means differed for each of the four tissue types. The trend for decreasing  $P_n$  with increasing acidity noted for current-year branch foliage is most likely a non-significant artifact.

f. Seasonal variation in acidic rain effect (A x M)

*One-year-old mature branch foliage* - The seasonal pattern of  $P_n$  by acidic rain treatment for one-year-old mature branch foliage is presented in Figure 18a. Regardless of acidic rain exposure, the seasonal pattern was similar to the general pattern described for one-year-old branch foliage in section 2.1. For most of the study period, foliage exposed to NAP had lower mean  $P_n$  than foliage exposed to either pH 5.1 or pH 3.0 acidic rain. Foliage exposed to pH 3.0 had the highest  $P_n$  in February, March and August through October. Foliage exposed to pH 5.1 had the greatest  $P_n$  among treatments for the months of April, May and July. Differences among treatment level means were not significant for any measurement period and the acidic rain x month interaction effect was not significant for the study as a whole ( $p=0.983$ , Table 16).

*Current-year mature branch foliage* - Regardless of acidic rain treatment, mean  $P_n$  of current-year branch foliage increased from June through November (Figure 18a). Although  $P_n$  for the pH 3.0 treatment tended to be slightly less than that for the NAP and pH 5.1 treatments from June through August, there were no significant treatment differences during any of the measurement periods. There was no significant acidic rain x month interaction effect on  $P_n$  for current-year branch foliage ( $p=0.989$ , Table 16).

*One-year-old seedling foliage* - There was a highly significant acidic rain x month interaction effect on  $P_n$  for one-year-old mature branch foliage ( $p=0.004$ , Table 17). Early in the study, mean  $P_n$  values were greatest for the pH 3.0 treatment and least for the NAP treatments. By May, this trend was reversed (Figure 18b). As with mature branches, there were no months in which significant differences among acidic rain treatment means were detected using Tukey's HSD comparison procedure at the  $p=0.05$  level.

*Current-year seedling foliage* - From June through November,  $P_n$  for current-year seedling foliage tended to be lowest for tissues exposed to pH 3.0 rain (Figure 18b). The relative ranking of  $P_n$  means between NAP and pH 5.1 treatments varied as values were greatest for pH 5.1 in June, August and November. Tissues exposed to NAP had the greatest mean  $P_n$  values in July, September and October. Differences among treatments were significant in July only as  $P_n$  for the NAP and pH 5.1 treatments were greater than that of the pH 3.0 treatment ( $p=0.05$ , Tukey's HSD). The variation in treatment mean rankings among months was significant as indicated by the significant month x acidic rain interaction term ( $p=0.001$ , Table 17).

*Lifestage and foliage age-class comparisons* - Seasonal variation of  $P_n$  by acid rain treatment differed little among lifestages for both current-year and one-year-old foliage. For current-year foliage, there was a greater variation in treatment level rankings among months for seedlings than for mature branches (Figures 18a and b). It should be noted

that there was significant month x acid rain interaction for both current-year and one-year-old seedling foliage and not for either age-class of mature branch foliage (Tables 16 and 17), suggesting greater acid rain effect on seasonal  $P_n$  for seedlings.

g. Interactive effect of acidic rain and genotype (A x G)

*One-year-old mature branch foliage* - There was no significant acidic rain x genotype interaction effect on  $P_n$  by one-year-old foliage of mature branches ( $p=0.104$ , Table 16). Treatment x genotype level means ranged from  $3.09 \mu\text{mol m}^{-2} \text{s}^{-1}$  for genotype 3088 under NAP to  $4.22 \mu\text{mol m}^{-2} \text{s}^{-1}$  for genotype 3087 exposed to pH 3.0 acidic rain (Table 20). Although RMANOVA did not indicate a significant interaction effect, the difference between the low and high treatment x genotype mean values was significant ( $p<0.05$ ) when tested using Tukey's HSD procedure. No other pair-wise differences were statistically significant.

*Current-year mature branch foliage* - Acidic rain x genotype level means for current-year mature branch foliage ranged from  $4.10 \mu\text{mol m}^{-2} \text{s}^{-1}$  (genotype 3088, pH 3.0) to  $4.85 \mu\text{mol m}^{-2} \text{s}^{-1}$  (genotype 3399, NAP) (Table 20). The acidic rain x genotype interaction effect was not significant ( $p=0.698$ , Table 16) and there were no significant differences among any of the acidic rain x genotype means.

*One-year-old seedling foliage* - Acidic rain x genotype interaction effects on  $P_n$  were not significant for one-year-old seedling foliage ( $p=0.655$ , Table 17). Mean  $P_n$  ranged from  $3.25 \mu\text{mol m}^{-2} \text{s}^{-1}$  (genotype 3088 at pH 3.0) to  $3.53 \mu\text{mol m}^{-2} \text{s}^{-1}$  (genotype 3087 at NAP) (Table 20).

*Current-year seedling foliage* - Acidic rain x genotype level means ranged from  $4.38 \mu\text{mol m}^{-2} \text{s}^{-1}$  to  $5.23 \mu\text{mol m}^{-2} \text{s}^{-1}$  for current-year seedling foliage (Table 20). There was no significant acidic rain x genotype interaction effect for current-year seedling foliage ( $p=0.612$ , Table 17).

*Lifestage and foliage age-class comparisons* - Regardless of life-stage or foliage age-class, there was no significant acidic rain x genotype interaction effect detected by RMANOVA.

h. Acidic rain application effect

The potential for a rain application effect on  $P_n$  independent from the effect of acidity exposure, was analyzed by comparing mean values (over all measurement dates) for seedlings and branches exposed to ambient air (AMB) and either the NAP or pH 5.1 acid rain treatments. This approach eliminates potential confounding that may arise from inclusion of the CF and 2xAMB ozone treatment effects and the acidity and nutrient effects of the pH 3.0 treatment.

*One-year-old mature branch foliage* - Mean  $P_n$  for one-year-old mature branch foliage exposed to the NAP and pH 5.1 treatments were 3.79 and 3.95  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively (Table 21). This difference among treatment means was not significant ( $p=0.510$ , Table 21).

*Current-year mature branch foliage* - The effect of simulated rain application on  $P_n$  of current-year mature branch foliage was not significant ( $p=0.784$ , Table 21). Mean  $P_n$  for the NAP and pH 5.1 treatments was 4.53 and 4.47  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively.

*One-year-old seedling foliage* - Mean  $P_n$  for one-year-old seedling foliage exposed to NAP was 3.62  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Table 20). For comparable foliage exposed to pH 5.1 acidic rain, mean  $P_n$  was 3.39  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (Table 21). The difference between the mean values were not significant ( $p=0.621$ , Table 21).

*Current-year seedling foliage* - Mean  $P_n$  for current-year seedling foliage exposed to NAP and pH 5.1 acidic rain was 5.13 and 4.97  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively (Table 21). The difference between the treatment means was not significant ( $p=0.621$ , Table 21).

*Lifestage and foliage age-class comparisons* - For all foliage types other than one-year-old branch tissue, mean  $P_n$  tended to be greater under the NAP exposure than under the pH 5.1 treatment but the differences among treatment means were statistically non-significant. This suggests that aside from possible effects due to acidity exposure, there was no real effect of rain application, or the lack of rain application, on  $P_n$  by any lifestage or foliage age-class.

*Comparison of rain application effect to acidic rain effect* - Significant acid rain main effects were absent (see sec. e) as were rain application effects. Yet, from the mean values observed, there tended to be lower  $P_n$  for tissue subjected to simulated rain than for tissue not receiving rain (NAP) for both age-classes of seedling foliage and for current-year mature branch foliage. The percent difference in  $P_n$  between NAP and pH 5.1 treatments ranged from -1.5 to -6.3 percent. When averaged over all ozone treatments and genotypes, the percent difference between NAP and pH 3.0 mean  $P_n$  values observed in the main effects analysis ranged from -3.5 to -9.6 percent. The magnitude of the differences in mean  $P_n$  between tissue exposed to NAP and rain of pH 5.1 or pH 3.0 are fairly similar. As a result, it is not possible to differentiate between treatment response to the application of rain solution and the acidity effect of the applied solution.

It is also interesting to note that  $P_n$  values for one-year-old branch foliage increased with solution application relative to NAP, regardless of whether the solution had a pH of 5.1 or a pH of 3.0. The observed increase was 4.2 percent when considering only pH 5.1 and AMB ozone conditions (sec. 2.3.4.1) or 7.3 and 8.7 percent for pH 5.1 and pH 3.0, respectively, when averaged over all ozone levels (sec. e). This also suggests that the effect may be due to solution application rather than the acidity of the applied solution.

i. Ozone effect (O)

*One-year-old mature branch foliage* - Net photosynthesis by one-year-old mature branch foliage declined with increasing ozone exposure as mean values for the CF, AMB and 2xAMB treatments were 4.05, 3.96, and 3.21  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively (Table 22). The effect of ozone was very highly significant ( $p=0.001$ , Table 16) as the reduction in  $P_n$  at 2xAMB, relative to AMB, was 19.0 percent.

*Current-year mature branch foliage* - There was also a trend for decreasing  $P_n$  with increasing ozone exposure for current-year mature branch foliage (Table 22) but the effect of ozone was not statistically significant ( $p=0.635$ , Table 16). Relative to AMB ozone,  $P_n$  for 2xAMB ozone was only 1.9 percent less.

*One-year-old seedling foliage* - There was an apparent decrease in  $P_n$  of 14.3 percent for one-year-old seedling foliage exposed to 2xAMB ozone, relative to tissue exposed to AMB ozone. Mean  $P_n$  decreased with increasing ozone exposure from 3.56  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for the CF treatment to 3.02  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for the 2xAMB treatment (Table 22). In spite of the substantial ozone-related decrease in  $P_n$ , the effect of ozone was not statistically significant ( $p=0.741$ , Table 17).

*Current-year seedling foliage* - Increasing ozone exposure resulted in a  $P_n$  decrease for current-year seedling foliage from 5.07  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in the CF treatment to 4.47  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in the 2xAMB treatment (Table 22). As with one-year-old foliage, the effect of ozone was not statistically significant ( $p=0.312$ , Table 17) even though the difference in  $P_n$  between the AMB and 2xAMB treatments was 9.6 percent.

*Lifestage and foliage age-class comparisons* - The response to ozone exposure was consistent for all foliage age-classes and lifestages as  $P_n$  decreased with increasing ozone exposure. The magnitude of the ozone effect, as measured by the percent difference in  $P_n$  between the AMB and 2xAMB exposures, varied from near 2 percent for current-year branch foliage to approximately 19 percent for one-year-old branch foliage. For both seedling and mature branch lifestages, the effect of ozone was greater for one-year-old foliage than for current-year foliage. This probably reflects the shorter period of exposure for current-year needles as they did not emerge until late-April or early-May.

j. Seasonal variation in ozone effect (O x M)

*One-year-old mature branch foliage* - The seasonal pattern of  $P_n$  by ozone treatment for one-year-old mature branch foliage is presented in Figure 19a. Over the study period,  $P_n$  values were consistently lowest for the 2xAMB treatment while the relative ranking of  $P_n$  for the CF and AMB treatments varied. The variation in ranking of treatment level means varied significantly among months as indicated by a significant month x ozone interaction term in the RMANOVA ( $p=0.006$ , Table 16). Means values for the 2xAMB treatment differed substantially ( $p<0.05$ , Tukey's HSD) from values for

the CF treatment in the months of April and May. Values for the 2xAMB treatment were significantly lower ( $p < 0.05$ , Tukey's HSD) than values for both the CF and AMB treatments in the months of September and October. The late season relative decrease in  $P_n$  under 2xAMB ozone was associated with a delay in late-season recovery of  $P_n$  for tissues exposed to elevated ozone (Figure 19a).

*Current-year mature branch foliage* - Regardless of ozone treatment,  $P_n$  of current-year mature branch foliage increased continuously from June through November (Figure 19a). There was some among-month variation in the relative ranking of ozone treatment means but from September through November, the highest  $P_n$  values were observed for the CF treatment and the lowest  $P_n$  values were observed for the 2xAMB treatment. The among-month variation was not statistically significant ( $p = 0.705$ , Table 16) and differences in treatment rankings were not significant ( $p = 0.05$ , Tukey's HSD) for any month.

*One-year-old seedling foliage* - With the exception of May,  $P_n$  values for one-year-old mature branch foliage were least for the 2xAMB treatment and very similar between the CF and AMB treatments (Figure 19b). Month x ozone interaction effects were non-significant ( $p = 0.315$ , Table 17) although among treatment differences did exist in the late growing season. Mean  $P_n$  for the 2xAMB treatment was less than that for the CF treatment in July and less than those for both the CF and AMB treatments in August, September and October ( $p < 0.05$ , Tukey's HSD).

*Current-year seedling foliage* - As with mature branch foliage, current-year seedling foliage demonstrated mean  $P_n$  values that were consistently lower for the 2xAMB treatment than for either the CF or AMB treatments (Figure 19b). The only exception to this generalization occurred in July when the mean value for the AMB treatment was atypically lower than that for the 2xAMB treatment. Despite mean values that were consistently more than 8 percent lower,  $P_n$  for the 2xAMB treatment was significantly lower than that for the AMB treatment only in November when the difference between mean values was 15.8 percent. The ozone x month interaction effect was not statistically significant ( $p = 0.343$ , Table 17).

*Lifestage and foliage age-class comparisons* - For all tissue types there was a tendency for relative decreases in  $P_n$  to occur in association with 2xAMB ozone exposure to be manifest in the later months of the study. The degree of significance varied among the tissue classes but the presence of an ozone effect was consistent as a 5 to 15 percent decrease for current-year tissue and as much as a 35 to 44 percent decrease for one-year-old tissue. The seasonal patterns for one-year-old tissue exposed to 2xAMB ozone were very similar to those exposed to CF or AMB treatments suggesting that elevated ozone had an impact on the magnitude of  $P_n$  without having substantial impact on the seasonality of  $P_n$ .

k. Interactive effect of ozone and genotype (O x G)

*One-year-old mature branch foliage* - When examined by individual genotype, there was a consistent response to ozone exposure for one-year-old mature branch foliage. For clones 3087, 3088 and 3399,  $P_n$  declined with increasing ozone exposure. The reduction in mean  $P_n$  under 2xAMB ozone, relative to AMB ozone, was 8.8, 22.8 and 25.6 percent, for genotypes 3087, 3088 and 3399, respectively (Table 23). The genotype x ozone interaction effect was not statistically significant ( $p=0.356$ , Table 16).

*Current-year mature branch foliage* - Current-year mature branch foliage of all three genotypes tended to have the lowest  $P_n$  when exposed to 2xAMB ozone (Table 23). While the greatest mean values for genotypes 3088 and 3399 were observed for the CF treatment, the greatest mean  $P_n$  value for genotype 3087 was observed for the AMB treatment. For all genotypes, there was little difference in  $P_n$  among the ozone treatments as mean values ranged from 4.34 to 4.46  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , from 4.17 to 4.32  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and from 4.61 to 4.85  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for genotypes 3087, 3088 and 3399, respectively. The genotype x ozone interaction effect was not significant for current-year mature branch foliage ( $p=0.987$ , Table 16).

*One-year-old seedling foliage* - There was a significant difference among genotypes in their response to ozone treatments for one-year-old seedling foliage ( $p=0.002$ , Table 17). The greatest mean  $P_n$  values for genotypes 3088 and 3399 were observed for the AMB ozone treatment, while the greatest mean  $P_n$  value for genotype 3087 occurred in the CF treatment (Table 23). For genotypes 3088 and 3399, tissue exposed to 2xAMB ozone had the lowest mean  $P_n$  while for genotype 3087, there was virtually no difference in  $P_n$  for the AMB and 2xAMB treatments (Table 23). Decreases in mean  $P_n$  under 2xAMB ozone, relative to ambient ozone, were 20.6 and 20.7 percent for genotypes 3088 and 3399, respectively. In contrast, the relative decrease in  $P_n$  for genotype 3087 was only 0.2 percent. These data indicate that a strong degree of variation in ozone sensitivity among the three half-sib seedling genotypes.

*Current-year seedling foliage* - There was a significant ozone x genotype interaction for current-year seedling foliage ( $p=0.024$ , Table 17). Mean  $P_n$  for genotypes 3087 and 3399 declined with increasing ozone exposure. Mean  $P_n$  for genotype 3087 ranged from 4.94  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for the CF treatment to 4.48  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 2xAMB ozone (Table 23). For genotype 3399, mean  $P_n$  ranged from 5.17  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for the CF treatment to 4.62  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 2xAMB ozone (Table 23). In contrast, the greatest mean  $P_n$ , 5.41  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , for genotype 3088 occurred in the AMB treatment. Mean values for genotype 3088 in the CF and 2xAMB treatment were 5.09 and 4.33  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively. The relative decrease in  $P_n$  between the AMB and 2xAMB exposures were 2.8, 20.1 and 4.1 percent for genotypes 3087, 3088 and 3399 respectively.

*Lifestage and foliage age-class comparison* - For all tissue types,  $P_n$  was decreased in the 2xAMB treatment, relative to the AMB and CF treatments. The magnitude of the ozone-related decrease varied significantly among genotypes for both age-classes of seedling foliage. Net photosynthesis of genotype 3087 seedling foliage of both age-classes was relatively insensitive to 2xAMB ozone. One-year-old foliage seedling foliage of genotype 3399 was sensitive to ozone while current-year foliage was not. In contrast, both age-classes of foliage demonstrated ozone sensitivity for seedlings of genotype 3088.

#### 1. Chamber effect: Ambient versus Non-chambered ambient

*One-year-old mature branch foliage* - The effect of chamber enclosure on  $P_n$  by one-year-old mature branch foliage was nearly significant ( $p=0.055$ , Table 24). Mean  $P_n$ , averaged over all genotypes, acid rain treatments and measurement dates, was  $3.96 \mu\text{mol m}^{-2} \text{s}^{-1}$  for AMB branches and  $3.62 \mu\text{mol m}^{-2} \text{s}^{-1}$  for NCAMB branches (Table 24).

*Current-year mature branch foliage* - Mean  $P_n$  for current-year mature branch foliage was 2.1 percent greater under chambered AMB conditions than under NCAMB conditions (Table 24). This difference in mean  $P_n$  was not statistically significant ( $p=0.721$ , Table 24).

*One-year-old seedling foliage* - Mean  $P_n$  of one-year-old seedling foliage was 12.0 percent greater for NCAMB than for AMB tissues (Table 24). This difference was nearly significant ( $p=0.068$ , Table 24).

*Current-year seedling foliage* - The difference in mean  $P_n$  between AMB and NCAMB treatments for current-year seedling foliage was not statistically significant ( $p=0.484$ , Table 24). The mean value for the NCAMB treatment was 4.4 percent less than the mean  $P_n$  value for the AMB treatment (Table 24).

*Lifestage and foliage age-class comparisons* - For current-year foliage of mature branches and seedlings, there was no substantial difference in  $P_n$  between tissues exposed to ambient ozone when enclosed in BECs or not enclosed in BECs. For these tissue classes, there was a tendency for mean  $P_n$  to be 2 to 9 percent greater under chambered conditions. In contrast, chamber enclosure had an effect on mean  $P_n$  for one-year-old seedling and branch foliage with the effect being positive for mature branches and negative for seedlings.

*Comparison of chamber effect to ozone effect* - The relative difference in  $P_n$  between tissues exposed to AMB and 2xAMB ozone treatments was -19.0, -1.9, -14.3 and -9.6 percent for one-year-old mature branch, current-year mature branch, one-year-old seedling and current-year seedling foliage types, respectively (Table 24). The effect of chamber enclosure for the same respective foliage types was +9.4, +2.1, -12.0 and +4.4 percent. Thus, the effects of ozone and chamber enclosure on  $P_n$  are of similar magnitude, but not always in the same direction. The analysis used in this report

uncouples the impacts of the two factors as all stated ozone effects are based on comparisons among chambered treatments. For purposes of extrapolation, given environmental conditions similar to those of this study, the difference in  $P_n$  estimates between AMB and NCAMB conditions suggest that absolute values in the field may be slightly higher for mature branch foliage and current-year seedling foliage, and lower for one-year-old seedling foliage.

m. Interactive effect of acidic rain and ozone (A x O)

*One-year-old mature branch foliage* - The interactive effects of ozone and acid rain on  $P_n$  were not significant ( $p=0.582$ , Table 16) for one-year-old mature branch foliage. Regardless of acid rain treatment, there was a consistent trend for the lowest mean  $P_n$  values to occur under the 2xAMB ozone exposure (Table 25).

*Current-year mature branch foliage* - Among ozone treatments, there was little variation in  $P_n$  for the NAP and pH 5.1 rain treatments. For tissues exposed to pH 3.0, there was a tendency for  $P_n$  to decrease with increasing ozone exposure from  $4.59 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the CF treatment to  $4.12 \mu\text{mol m}^{-2} \text{s}^{-1}$  in the 2xAMB treatment (Table 25). This apparent difference in response to ozone among acid rain treatments was not statistically significant ( $p=0.690$ , Table 16).

*One-year-old seedling foliage* - The interactive effects of ozone and acid rain on  $P_n$  were not statistically significant for one-year-old seedling foliage ( $p=0.990$ , Table 17). Regardless of acidic rain treatment, there was a tendency for mean  $P_n$  to be lower for tissue exposed to 2xAMB ozone than for tissues exposed to either CF or AMB ozone (Table 25). The decrease in  $P_n$  under 2xAMB ozone, relative to AMB ozone, was 11, 14 and 18 percent for the NAP, pH 5.1 and the pH 3.0 acidic rain levels, respectively (Table 25).

*Current-year seedling foliage* - Among acid rain treatments, there was some variation in  $P_n$  response to ozone for current-year seedling foliage. For the NAP and pH 5.1 treatments, there was a tendency for  $P_n$  to decrease with increasing ozone exposure. Relative to values for the CF treatment, values for the 2xAMB treatment were 22 and 6 percent less for the NAP and pH 5.1 rain treatments, respectively (Table 25). For tissue exposed to pH 3.0, there was little variation in  $P_n$  among ozone levels as the greatest mean  $P_n$  was observed for the AMB ozone exposure and the difference in  $P_n$  between the CF and 2xAMB ozone treatments was 5 percent (Table 25). This variation in response to ozone among acid rain treatments was not significant ( $p=0.711$ , Table 17).

*Lifestage and foliage age-class comparisons* - Regardless of lifestage or foliage age-class, there were no statistically significant acidic rain x ozone interaction effects on  $P_n$ .

n. Seasonal variation in the interactive effect of acidic rain and genotype  
(M x A x G)

There was a significant ( $p > 0.011$ ) acidic rain x genotype x month interaction effect on  $P_n$  for one-year-old seedling foliage (Table 17). The nature of the interaction is difficult to resolve but can be seen in Figures 20a-c. Among genotypes, there was variation in the ranking of  $P_n$  means for the NAP, pH 5.1 and pH 3.0 acidic rain treatments, particularly from June through October. There was a substantial increase in mean  $P_n$  from June to July for seedlings of genotypes 3087 and 3399 that were exposed to the NAP treatment. This increase was not evident for seedlings of genotype 3088 exposed to the NAP treatment. From July through October, the greatest  $P_n$  values for both genotypes 3087 and 3399, were observed for seedlings exposed to the NAP treatment while seedlings exposed to the pH 3.0 treatment tended to have the lowest. In contrast, there was no clear late season differentiation of mean  $P_n$  among acidic rain treatments for genotype 3088 seedlings. These results imply that there was a genotypic difference in response to acidic rain treatment, yet for all genotypes, the degree of photosynthetic response to the acidic rain treatments was relatively low.

Table 16. Summary of mature branch mid-day photosynthesis repeated measures ANOVA.

Mature Branch Photosynthesis RMANOVA								
Source	Current-year Foliage				One-year-old Foliage			
	DF	MS	F	Pr>F	DF	MS	F	Pr>F
Between Subj.								
Acid Rain (A)	2	0.92	0.69	0.526	2	4.50	2.14	0.174
Genotype (G)	2	6.22	4.66	0.041	2	14.44	6.89	0.015
A x G	4	0.75	0.56	0.698	4	5.53	2.64	0.104
Error I	9	1.33			9	2.10		
Within Subj.								
Ozone (O)	2	0.60	0.47	0.635	2	34.65	17.81	0.001
A x O	4	0.73	0.57	0.690	4	1.43	0.73	0.582
G x O	4	0.12	0.10	0.982	4	2.28	1.17	0.356
A x G x O	8	1.31	1.02	0.457	8	1.90	0.98	0.485
Error II	18	1.29			18	1.95		
Month (M)	5	50.41	29.02	<0.001	8	38.77	21.61	<0.001
M x A	10	0.43	0.25	0.989	16	0.69	0.38	0.983
M x G	10	2.52	1.45	0.190	16	2.64	1.47	0.135
M x A x G	20	1.04	0.60	0.892	32	1.36	0.76	0.803
Error III	45	1.74			72	1.79		
M x O	10	0.50	0.67	0.705	16	1.94	2.26	0.006
M x A x O	20	0.87	1.17	0.318	32	1.09	1.27	0.170
M x G x O	20	0.42	0.56	0.893	32	0.99	1.15	0.285
M x A x G x O	40	0.65	0.87	0.651	64	0.85	0.99	0.504
Error IV	90	0.74			144	0.86		

Table 17. Summary of seedling mid-day photosynthesis repeated measures ANOVA.

Seedling Photosynthesis RMANOVA								
Source	Current-year Foliage				One-year-old Foliage			
	DF	MS	F	Pr>F	DF	MS	F	Pr>F
Between Subj.								
Acid Rain (A)	2	15.75	2.63	0.126	2	1.94	0.13	0.880
Ozone (O)	2	7.93	1.33	0.312	2	4.59	0.31	0.741
A x O	4	3.20	0.54	0.711	4	1.05	0.07	0.990
Error I	9	5.98			9	14.90		
Within Subj.								
Genotype (G)	2	3.02	2.29	0.130	2	0.74	0.62	0.547
A x G	4	0.90	0.68	0.612	4	0.74	0.62	0.655
O x G	4	4.82	3.65	0.024	4	7.52	6.32	0.002
A x O x G	8	1.77	1.34	0.287	8	1.52	1.28	0.315
Error II	18	1.32			18	1.19		
Month (M)	5	29.60	13.27	<0.001	7	64.31	21.26	<0.001
M x A	10	8.57	3.84	0.001	14	8.11	2.68	0.004
M x O	10	2.48	1.11	0.376	14	3.43	1.14	0.343
M x A x O	20	2.85	1.28	0.241	28	1.53	0.51	0.974
Error III	45	2.23			63	3.03		
M x G	10	2.50	2.26	0.021	14	0.59	0.79	0.676
M x A x G	20	1.40	1.26	0.226	28	1.38	1.86	0.011
M x O x G	20	0.93	0.84	0.662	28	0.79	1.06	0.392
M x A x O x G	40	1.57	1.42	0.086	56	0.85	1.14	0.267
Error IV	90	1.11			126	0.74		

Table 18. Net photosynthesis ( $P_n$ ) by genotype for one-year-old and current-year foliage of mature branches and seedlings of *Pinus ponderosa*. Values are means and standard errors of the means calculated over all measurement dates, ozone treatments and acid rain treatments. For each lifestage and foliage age-class combination, mean values followed by a common letter do not differ at the  $p=0.05$  probability level.

Net Photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) by Genotype					
Lifestage	Foliage Age-class		Genotype		
			3087	3088	3399
Mature Branch	1991	mean	4.001a	3.418b	3.795ab
		s.e.	0.169	0.152	0.100
Mature Branch	1992	mean	4.399ab	4.242b	4.713a
		s.e.	0.109	0.088	0.108
Seedling	1991	mean	3.354a	3.416a	3.330a
		s.e.	0.107	0.109	0.113
Seedling	1992	mean	4.674a	4.943a	4.869a
		s.e.	0.116	0.154	0.131

Table 19. Net photosynthesis ( $P_n$ ) by acidic rain treatment for one-year-old and current-year foliage of mature branches and seedlings of *Pinus ponderosa*. Acid rain treatments include no acid rain (NAP), pH 5.1 simulated rain (pH 5.1) and pH 3.0 simulated rain (pH 3.0). Values are means and standard errors of the means calculated over all measurement dates, ozone treatments and genotypes. For each lifestage and foliage age-class combination, mean values followed by a common letter do not differ at the  $p=0.05$  probability level.

Net Photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) by Acidic Rain Treatment					
Lifestage	Foliage Age-class	Acidic Rain Treatment			
			NAP	pH 5.1	pH 3.0
Mature Branch	1991	mean	3.550a	3.808a	3.861a
		s.e.	0.186	0.145	0.175
Mature Branch	1992	mean	4.546a	4.446a	4.362a
		s.e.	0.123	0.118	0.148
Seedling	1991	mean	3.463a	3.296a	3.341a
		s.e.	0.150	0.200	0.128
Seedling	1992	mean	4.960a	5.039a	4.487a
		s.e.	0.219	0.152	0.168

Table 20. Net photosynthesis by acid rain treatment and genotype for one-year-old and current-year foliage of mature branch and seedling foliage of *Pinus ponderosa*. Acid rain treatments include no acid rain (NAP), pH 5.1 simulated rain (pH 5.1) and pH 3.0 simulated rain (pH 3.0). Genotypes include 3087, 3088 and 3399. Values are means and standard errors of the means calculated over all measurement dates and ozone treatments. For each lifestage and foliage age-class combination, mean values followed by a common letter do not differ at the p=0.05 probability level.

Net Photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) by Acid Rain Treatment and Genotype						
Parameter	NAP		pH 5.1		pH 3.0	
	3087	3088	3399	3087	3088	3399
Mature Branch One-year-old Foliage						
mean	3.641ab	3.086b	3.923ab	4.159ab	3.772ab	3.493ab
s.e.	0.352	0.160	0.088	0.065	0.291	0.052
Mature Branch Current-year Foliage						
mean	4.485a	4.303a	4.852a	4.234a	4.324a	4.778a
s.e.	0.004	0.005	0.261	0.106	0.166	0.012
Seedling One-year-old Foliage						
mean	3.526a	3.470a	3.394a	3.288a	3.306a	3.294a
s.e.	0.180	0.188	0.211	0.231	0.212	0.244
Seedling Current-year Foliage						
mean	4.822a	5.228a	4.829a	4.824a	5.119a	5.174a
s.e.	0.247	0.291	0.241	0.160	0.197	0.219
				4.375a	4.483a	4.602a
				0.171	0.271	0.205

Table 21. Net photosynthesis for one-year-old and current-year foliage of mature branch and seedling foliage of *Pinus ponderosa* under ambient ozone and exposed to either no acid rain (NAP) or pH 5.1 rain (pH 5.1). Values are means and standard errors of the means calculated over all measurement dates and genotypes. For each lifestage and foliage age-class combination, probability values indicate the likelihood that differences between means do not differ significantly at the  $p=0.05$  level.

Effect of Acid Rain Application on Net Photosynthesis				
Parameter	Net Photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )		t-test	
	NAP	pH 5.1	t value	P<t
Mature Branch One-year-old Foliage				
mean	3.791	3.951	0.684	0.510
s.e.	0.208	0.107		
Mature Branch Current-year Foliage				
mean	4.533	4.465	0.281	0.784
s.e.	0.140	0.197		
Seedling One-year-old Foliage				
mean	3.617	3.390	0.503	0.633
s.e.	0.374	0.235		
Seedling Current-year Foliage				
mean	5.129	4.965	0.520	0.621
s.e.	0.176	0.262		

Table 22. Net photosynthesis by ozone treatment for one-year-old and current-year foliage of mature branches and seedlings of *Pinus ponderosa*. Ozone treatments include charcoal filtered ambient air (CF), ambient air (AMB) and air supplemented with ozone to twice ambient ozone concentration (2xAMB). Values are means and standard errors of the means calculated over all measurement dates, acidic rain treatments and genotypes. For each lifestage and foliage age-class combination, mean values followed by a common letter do not differ at the p=0.05 probability level.

Net Photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) by Ozone Treatment					
Lifestage	Foliage Age-class		Ozone Treatment		
			CF	AMB	2xAMB
Mature Branch	1991	mean	4.052a	3.959a	3.208b
		s.e.	0.148	0.094	0.147
Mature Branch	1992	mean	4.522a	4.459a	4.374a
		s.e.	0.128	0.087	0.111
Seedling	1991	mean	3.561a	3.519a	3.016a
		s.e.	0.153	0.171	0.148
Seedling	1992	mean	5.065a	4.947a	4.474a
		s.e.	0.224	0.143	0.245

Table 23. Net Photosynthesis by ozone treatment and genotype for one-year-old and current-year foliage of mature branch and seedling foliage of *Pinus ponderosa*. Ozone treatments include charcoal-filtered (CF), ambient (AMB) and twice ambient (2xAMB) atmospheric ozone concentration. Genotypes include 3087, 3088 and 3399. Values are means and standard errors of the means calculated over all measurement dates and ozone treatments. For each life stage and foliage age-class combination, mean values followed by a common letter do not differ at the  $p=0.05$  probability level.

Net Photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) by Ozone Treatment and Genotype									
Parameter	CF			AMB			2xAMB		
	3087	3088	3399	3087	3088	3399	3087	3088	3399
Mature Branch One-year-old Foliage									
mean	4.192a	3.741a	4.223a	4.096a	3.675a	4.106a	3.733a	2.836a	3.056a
s.e.	0.262	0.284	0.210	0.100	0.161	0.176	0.201	0.186	0.240
Mature Branch Current-year Foliage									
mean	4.401a	4.317a	4.848a	4.456a	4.239a	4.680a	4.338a	4.171a	4.612a
s.e.	0.167	0.235	0.226	0.103	0.131	0.173	0.187	0.205	0.167
Seedling One-year-old Foliage									
mean	3.559ab	3.621ab	3.514ab	3.255ab	3.694a	3.614ab	3.249ab	2.930b	2.866b
s.e.	0.197	0.139	0.200	0.187	0.135	0.186	0.174	0.207	0.134
Seedling Current-year Foliage									
mean	4.937ab	5.088ab	5.170ab	4.608ab	5.412a	4.820ab	4.476ab	4.330b	4.615ab
s.e.	0.240	0.206	0.284	0.133	0.185	0.195	0.207	0.306	0.174

Table 24. Net photosynthesis for one-year-old and current-year foliage of mature branch and seedling foliage of *Pinus ponderosa* exposed to ambient ozone either in BECs (AMB) or as non-chambered companion tissues (NCAMB). Values are means and standard errors of the means calculated over all acid rain treatments, genotypes and measurement dates. For each lifestage and foliage age-class combination, probability values indicate the likelihood that differences between mean do not differ significantly at the  $p=0.05$  level.

Chamber Effect on Net Photosynthesis: Ambient versus Non-chambered Companions						
Lifestage	Foliage Age-class	Parameter	Net Photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )		T-test	
			AMB	NCAMB	T value	P>t
Branch	1991	mean	3.959a	3.620a	1.984	0.055
		s.e.	0.094	0.143		
Branch	1992	mean	4.459a	4.368a	0.555	0.583
		s.e.	0.087	0.138		
Seedling	1991	mean	3.520a	4.001a	1.957	0.068
		s.e.	0.148	0.179		
Seedling	1992	mean	4.947a	4.761a	0.912	0.375
		s.e.	0.123	0.150		

Table 25. Net photosynthesis by acid rain treatment and ozone treatment for one-year-old and current-year foliage of mature branch and seedling foliage of *Pinus ponderosa*. Acid rain treatments include no acid rain (NAP), pH 5.1 simulated rain (pH 5.1) and pH 3.0 simulated rain (pH 3.0). Ozone treatments include charcoal filtered (CF), ambient (AMB), and twice ambient (2xAMB) ozone concentrations. Values are means and standard errors of the means calculated over all measurement dates and ozone treatments. For each life stage and foliage age-class combination, mean values followed by a common letter do not differ at the  $p=0.05$  probability level.

Net Photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) by Acid Rain Treatment and Ozone Treatment								
Parameter	NAP			pH 5.1			pH 3.0	
	CF	AMB	2xAMB	CF	AMB	2xAMB	CF	AMB 2xAMB
Mature Branch One-year-old Foliage								
mean	3.706a	3.791a	3.153a	4.315a	3.951a	3.158a	4.135a	4.136a 3.314a
s.e.	0.237	0.208	0.236	0.230	0.107	0.303	0.272	0.155 0.267
Mature Branch Current-year Foliage								
mean	4.548a	4.533a	4.558a	4.427a	4.465a	4.446a	4.591a	4.378a 4.116a
s.e.	0.198	0.140	0.207	0.219	0.197	0.137	0.277	0.129 0.205
Seedling One-year-old Foliage								
mean	3.547a	3.617a	3.222a	3.574a	3.390a	2.929a	3.574a	3.547a 2.896a
s.e.	0.203	0.374	0.188	0.399	0.235	0.391	0.063	0.194 0.198
Seedling Current-year Foliage								
mean	5.473a	5.129a	4.277a	5.245a	4.965a	4.905a	4.476a	4.745a 4.239a
s.e.	0.211	0.176	0.446	0.348	0.262	0.210	0.380	0.202 0.289

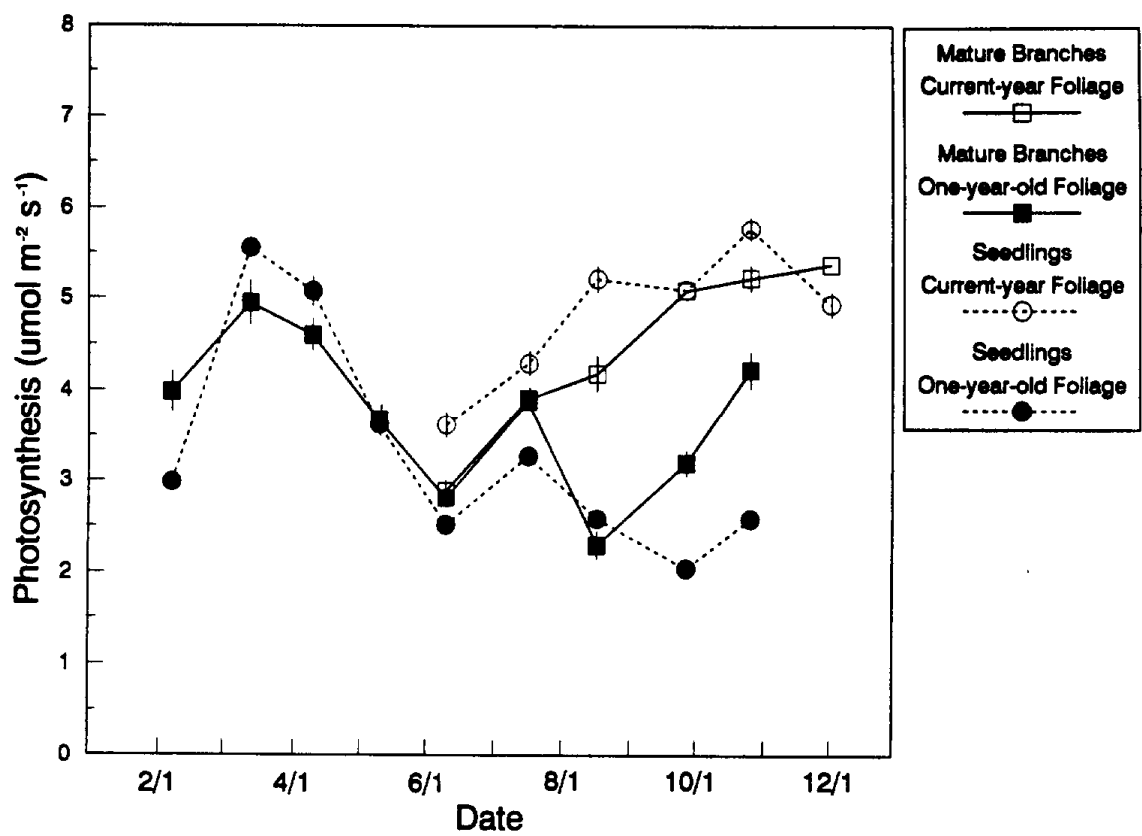


Figure 15. Seasonal variation in net photosynthesis by current-year (1992) and one-year-old (1991) foliage of *Pinus ponderosa* in mature branches and seedlings. Values are means calculated over all genotypes and pollutant treatments. Vertical lines represent one standard error of the mean.

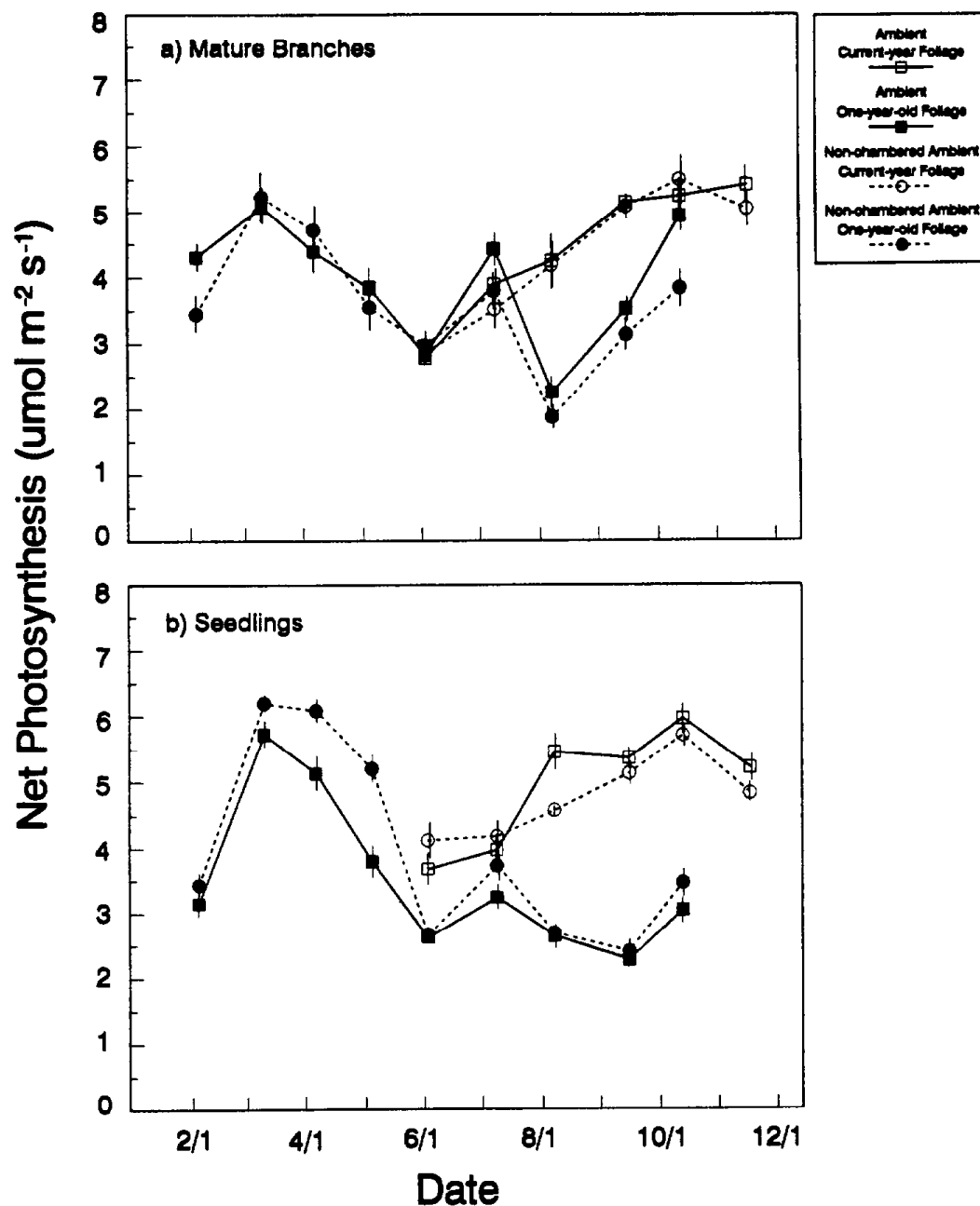


Figure 16. Seasonal variation in mid-day net photosynthesis of current-year and one-year-old foliage of *Pinus ponderosa* a) mature branches and b) seedlings exposed to ambient ozone in BECs (AMB) or non-chambered ambient ozone (NCAMB). Values are means over all genotypes and acidic rain treatments. Vertical lines represent one standard error of the mean.

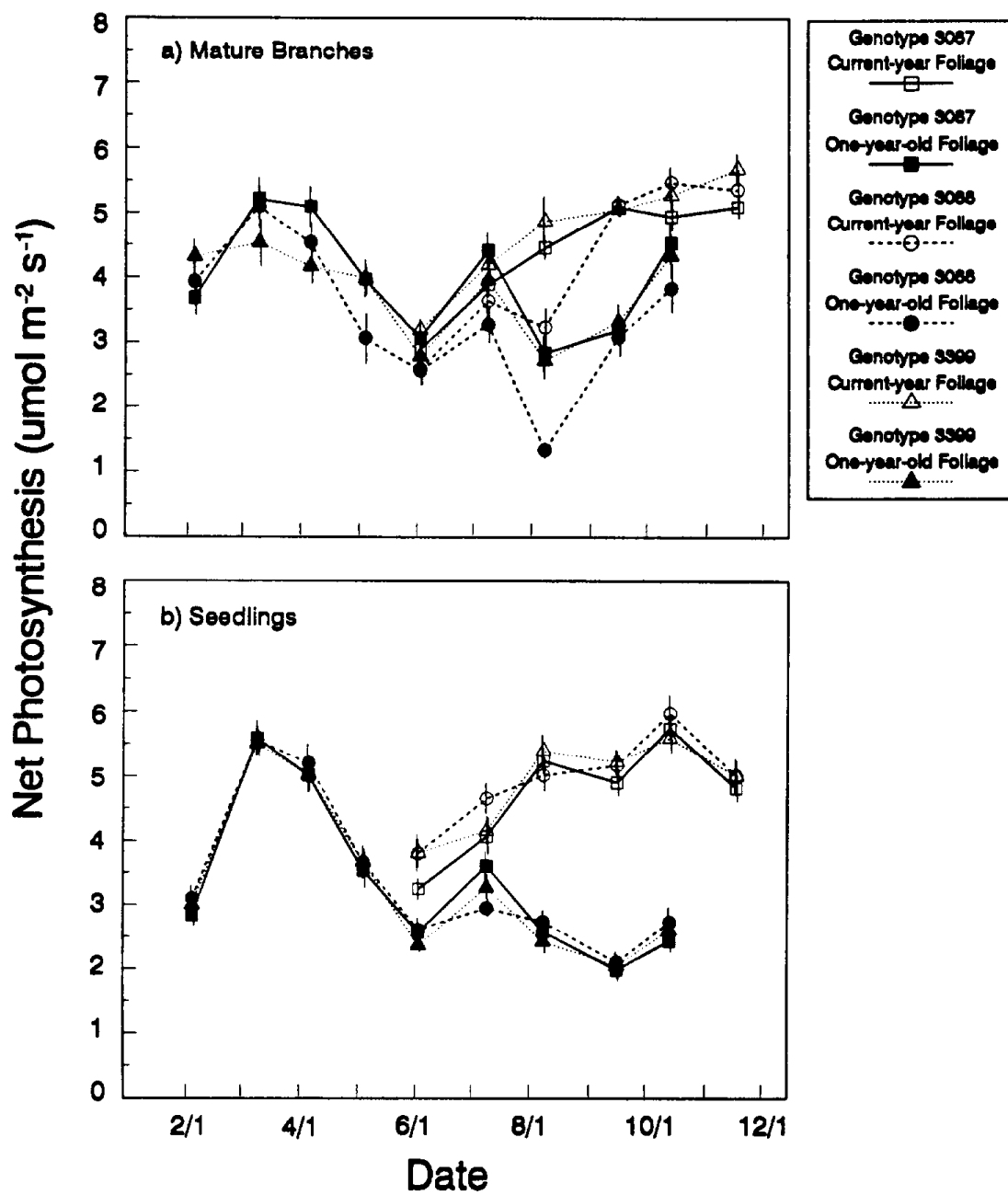


Figure 17. Seasonal variation in mid-day net photosynthesis for current-year and one-year-old foliage of *Pinus ponderosa* a) mature branches and b) seedlings of genotypes 3087, 3088 and 3399. Values are means over all acidic rain and ozone treatments. Vertical lines represent one standard error of the mean.

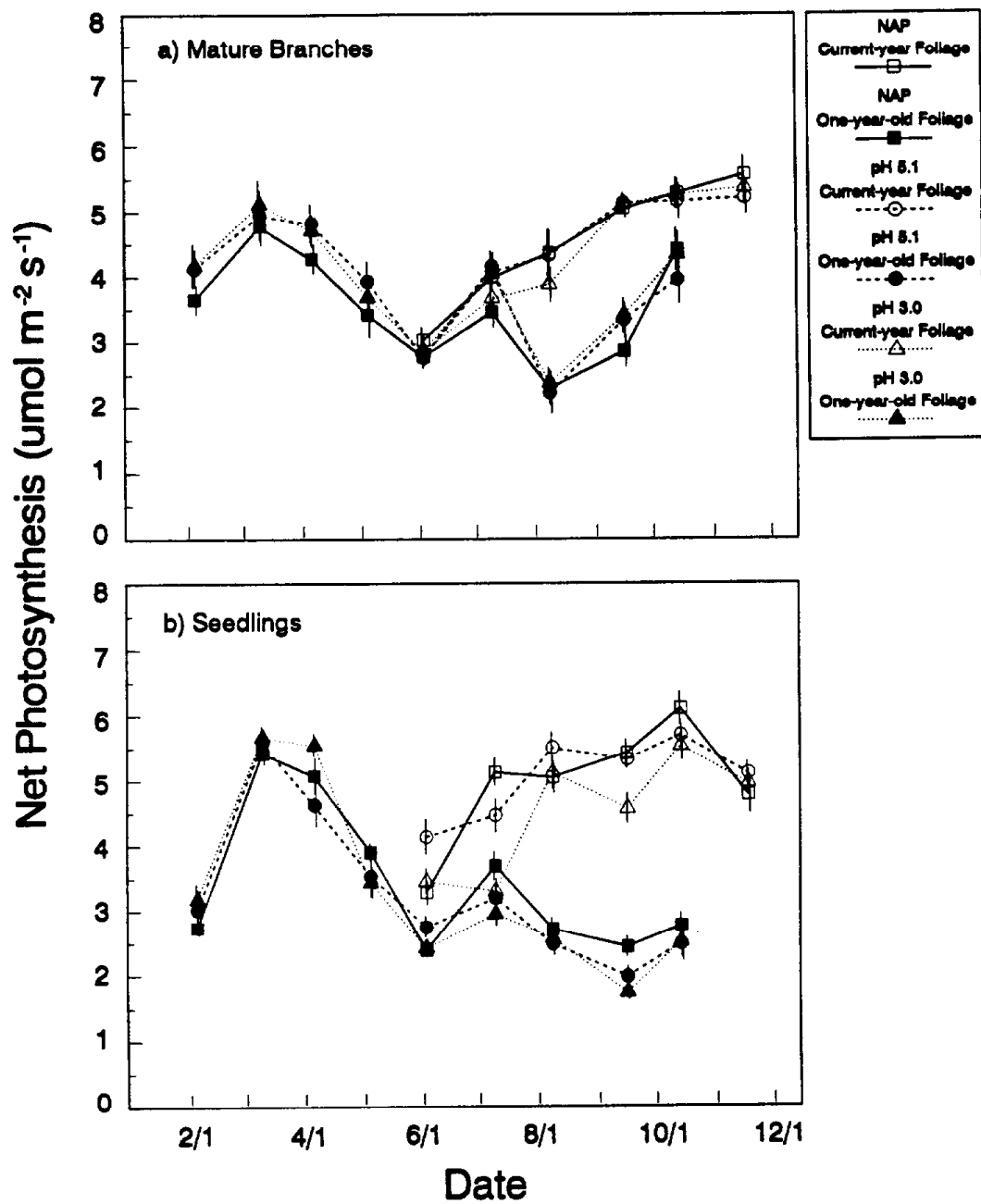


Figure 18. Seasonal variation in mid-day net photosynthesis for current-year and one-year-old foliage of *Pinus ponderosa* a) mature branches and b) seedlings exposed to no acid rain (NAP), pH 5.1 rain (pH 5.1) or pH 3.0 rain (pH 3.0). Values are means over all genotypes and ozone treatments. Vertical lines represent one standard error of the mean.

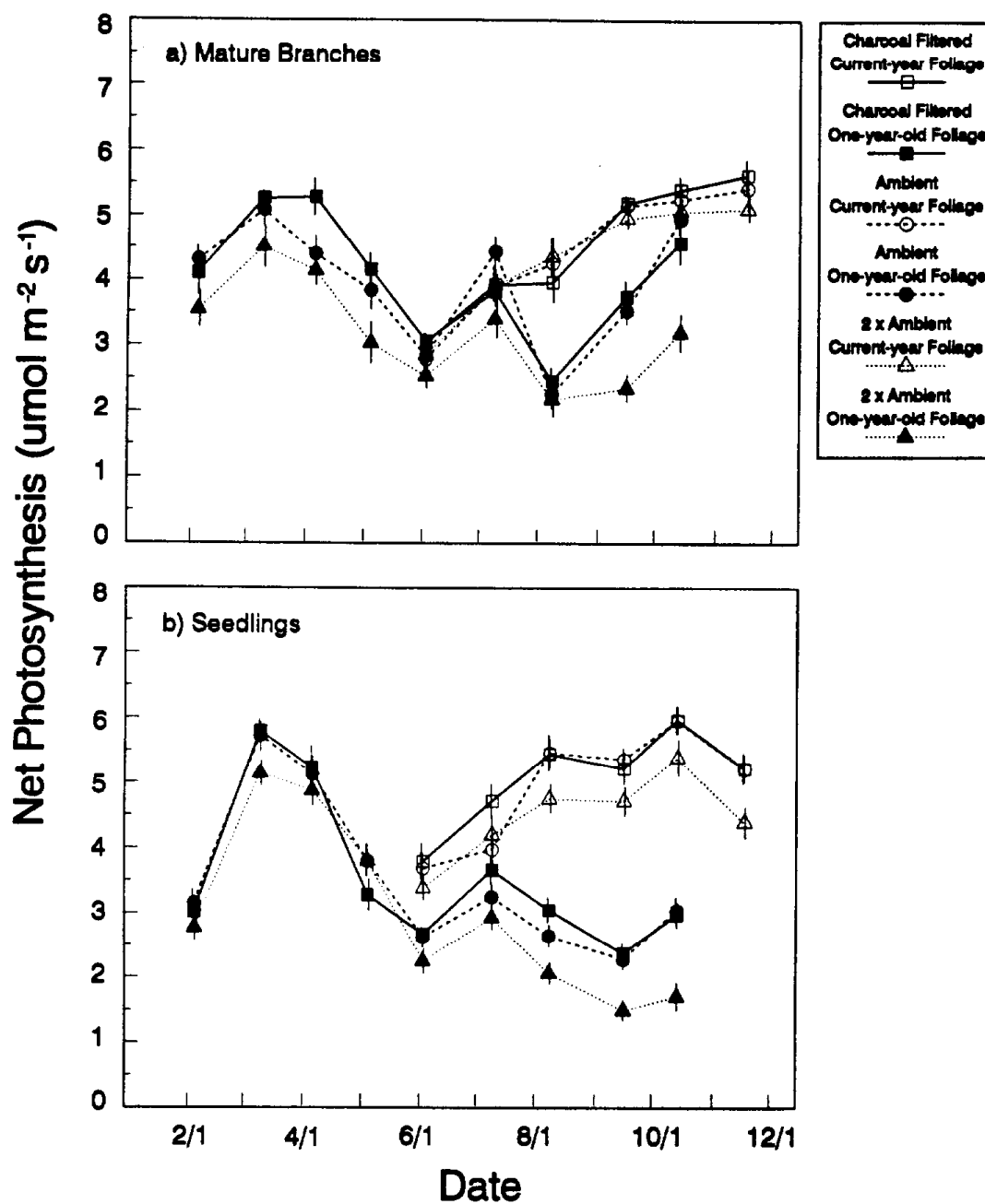


Figure 19. Seasonal variation in mid-day net photosynthesis for current-year and one-year-old foliage of *Pinus ponderosa* a) mature branches and b) seedlings exposed to charcoal-filtered air (CF), ambient ozone (AMB) or twice ambient ozone (2xAMB) in BECs. Values are means over all genotypes and ozone treatments. Vertical lines represent one standard error of the mean.

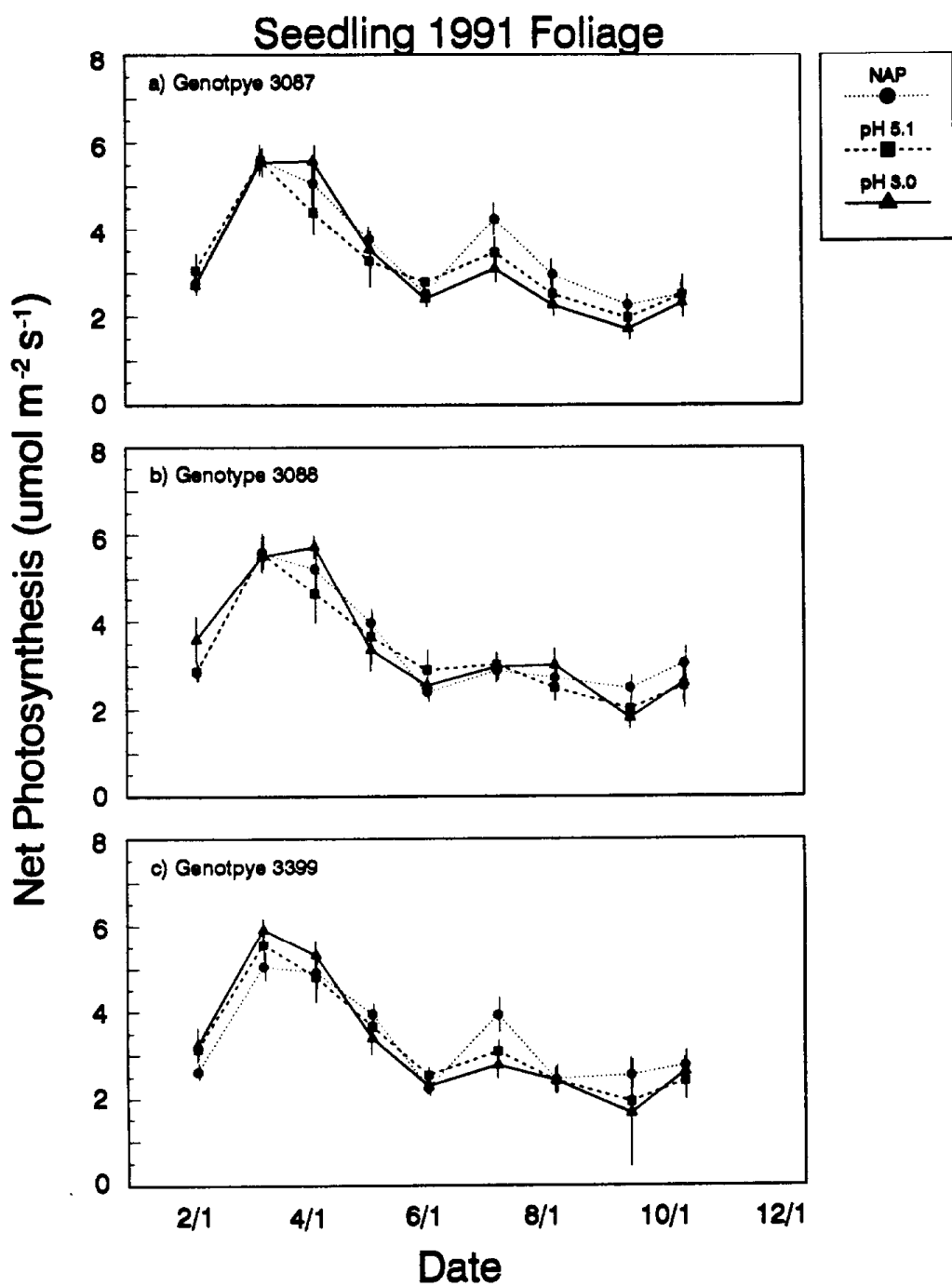


Figure 20. Seasonal variation in mid-day net photosynthesis for one-year-old foliage *Pinus ponderosa* half-sib seedlings genotypes exposed to no acid rain (NAP), pH 5.1 rain or pH 3.0 rain. Values are means over all ozone treatments for genotypes a) 3087, b) 3088, and c) 3399. Vertical lines represent one standard error of the mean.

## D. Gas-exchange Environmental Response Surfaces

Analysis of covariance was used to identify significant effects of acidic rain, ozone, genotype, foliage age-class and their interactions from the response surface data for seedlings and mature branches collected in late-summer (August-September) and fall (November). The ANACOV was also used to determine which environmental parameters (light intensity, cuvette temperature and vapor pressure deficit) accounted for significant portions of the observed variability in photosynthesis and stomatal conductance rates. Several families of response surface models were fit to the data with each family representing a significant main or interaction effect indicated by ANACOV as having a significant effect on gas-exchange response to environment. Each family of models consisted of response surfaces representing each treatment level of the main or interaction effect under evaluation.

The model parameter estimates for each family of fitted response surfaces are presented as a series of tables in Appendix B. As indicated by the coefficients of determination ( $R^2$ ), the fitted response surface models for mature branches accounted for 35 to 92 percent of the variation in  $P_n$ , while those for seedlings accounted for 16 to 92 percent of the variation in  $P_n$ . The  $g_s$  response surface models accounted for 40 to 62 percent of observed variation for branches and from 16 to 90 percent for seedlings. Bonferonni confidence intervals were calculated for each response model term to identify significantly different response surfaces within a family of fitted surfaces. Effects identified by ANACOV as being significant sources of variation did not consistently have significantly different coefficient estimates when tested by the Bonferonni method. This may have been the result of the conservative nature of the Bonferonni statistic in controlling the error rate for the family of comparisons to be made.

### 1. Stomatal Conductance

There were no significant ozone, genotype or age-class main effects for mature branches in late-summer or fall and no significant acidic rain main effect for branches in the fall indicated by ANACOV (Tables 26). Seedling  $g_s$  response surface models fitted to the late-summer data accounted for a very low proportion of the observed variation and will not be presented in the results which follow. Of the environmental variables, light intensity and vapor pressure deficit tended to have significant effect on mature branch  $g_s$  while cuvette temperature was only significant as an interacting factor with light intensity and vapor pressure deficit (Table 26). Stomatal conductance of seedling foliage responded to cuvette temperature and vapor pressure deficit in the fall but only vapor pressure deficit in the late-summer (Table 27).

#### a. Acidic rain effect (A)

Acidic rain had a significant effect on mature branch  $g_s$  in late-summer ( $p=0.041$ , Table 26) but not in the fall ( $p=0.103$ , Table 26). In late-summer,  $g_s$  of mature branches

exposed to pH 5.1 rain increased with increasing temperature at both high and low light intensities (Figure 21a). For branches exposed to the pH 3.0 treatment,  $g_s$  peaked at temperatures between 30 and 35 °C under low light conditions but continuously increased with increasing temperature under high light intensity conditions (Figure 21b). At low cuvette temperatures,  $g_s$  decreased with increasing light intensity for branches in the pH 3.0 treatment and increased with increasing light intensity for branches in the pH 5.1 treatment (Figures 21a and 21b).

#### b. Interactive effect of acidic rain and genotype (A x G)

Seedling stomatal conductance was significantly influenced by an acidic rain x genotype interaction in both the late-summer and fall measurement periods ( $p=0.013$  and  $p=0.001$ , respectively). Seedlings of genotype 3087 in both pH 5.1 and pH 3.0 rain treatments demonstrated a weak  $g_s$  response to light at all temperatures. Conductance response to temperature differed between the two rain treatments; for seedlings exposed to pH 5.1, increasingly greater temperatures were associated with higher values of  $g_s$ . For seedlings of clone 3087 exposed to pH 3.0,  $g_s$  values were maximum at approximately 25 °C (Figures 22a and 22b). Conductance responses to cuvette temperature were similar for seedlings of genotype 3088 exposed to pH 5.1 and pH 3.0 rain treatments. At low temperatures,  $g_s$  for seedlings of genotype 3088 in both pH 5.1 and pH 3.0 treatments tended to decrease with light intensities in excess of approximately 500  $\mu\text{E m}^{-2} \text{ s}^{-1}$  (Figures 22c and 22d). Stomatal conductance of genotype 3399 seedlings increased with both increasing temperature and increasing light intensity. At low temperatures,  $g_s$  values were slightly greater for seedlings exposed to the pH 5.1 treatment. However, at high light intensities,  $g_s$  values for seedlings of genotype 3399 were greater in the pH 3.0 treatment (Figures 22e and 22f). It should be noted that although the response surface forms for genotype 3399 are unusual, they are based on models that have a relatively strong fit to the measured data ( $R^2$  values of 0.532 and 0.601 for pH 5.1 and pH 3.0, respectively).

## 2. Net Photosynthesis

There was a significant  $P_n$  response to the ozone main effect for mature branches and a significant foliage age-class main effect for both mature branches and seedlings in late summer (Tables 26 and 27). The effects of acidic rain and genotype on  $P_n$  was manifest as significant interaction terms. Light intensity and cuvette temperature had the most highly significant effects on mature branch  $P_n$  in the late-summer while light intensity, cuvette temperature and vapor pressure deficit all had strong effects on mature branch  $P_n$  measured in November (Table 26). Seedling photosynthesis rates were influenced by light intensity and cuvette temperature while vapor pressure deficit had no significant effect in either measurement period (Table 27).

a. Ozone effect (O)

For mature branches, ANACOV indicated a significant ozone effect on photosynthesis for both the late-summer and fall measurement periods ( $p=0.085$  and  $p=0.009$ , respectively). In late-summer,  $P_n$  of mature branches exposed to 2xAMB ozone was decreased relative to  $P_n$  of mature branches exposed to the AMB treatment (Figures 23a and 23b). The relative decrease was greatest for conditions of low temperature and high light intensity (Figures 23a and 23b).

b. Interactive effect of ozone and genotype (O x G)

The analysis also suggested a slight ozone x genotype interaction effect on mature branch photosynthesis in November ( $p=0.059$ , Table 26). For genotype 3087, there was only a slight reduction in  $P_n$  for tissues exposed to 2xAMB ozone relative to those exposed to AMB ozone (Figures 24a and 24b). There was little difference in the form of the  $P_n$  response surfaces between AMB and 2xAMB treatments for branches of genotype 3087. For genotype 3088, there was also little difference in peak  $P_n$  values for the AMB and 2xAMB response surfaces, yet the response to temperature and light differed substantially between surfaces for the two treatments. Under low light intensities,  $P_n$  values were negligible (actually negative but represented in the figures as zero for purposes of clarity) when temperatures exceeded approximately 27 and 29 °C for the 2xAMB and AMB levels, respectively (Figures 24c and 24d). For mature branches of genotype 3399, peak  $P_n$  values differed markedly among ozone treatments. Whereas  $P_n$  values for branches under AMB ozone peaked between 20 and 25 °C at low light intensities, and between 25 and 30 °C at high light intensities,  $P_n$  measured in mature branches under 2xAMB ozone tended to increase with increasing temperature over the entire 15 to 35 °C range (Figures 24e and 24f).

c. Foliage age-class effect (C)

Peak values of  $P_n$  measured in late-summer were approximately 7.5 and 5.0  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for current-year and one-year-old mature branch foliage, respectively. In spite of higher maximum rates for current-year foliage,  $P_n$  rates for one-year-old foliage were substantially greater under low light intensity conditions (Figures 25a and 25b). This highly significant differences in  $P_n$  between current-year and one-year-old foliage was observed for the late-summer period but not in November (Table 26).

Seedling  $P_n$  rates differed significantly between foliage-age-classes in the late-summer ( $p<0.001$ ). Peak  $P_n$  values for one-year-old foliage were only 30-40 percent of those for current-year foliage. The amplitude of  $P_n$  rates over the temperature range of 15 to 35 °C and the light intensity range of 0 to 1200  $\mu\text{E m}^{-2} \text{s}^{-1}$  was very limited for one-year-old tissue indicating a general lack of temperature and light intensity response (Figure 26b).

d. Interactive effect of acidic rain, ozone and genotype (A x O x G)

In the fall, seedling photosynthesis was significantly affected by an acid rain x ozone x genotype interaction ( $p=0.045$ ) with ozone treatment and genotype being the dominant sources of variation (Table 27). For half-sib seedlings of clone 3087, peak  $P_n$  values were greater for the AMB ozone treatment than for the 2xAMB ozone treatment. Seedlings from both ozone treatments had similar  $P_n$  responses to light intensity but seedlings in the 2xAMB treatment demonstrated a lack of temperature response (Figures 27a and 27b). Seedlings of clone 3088 also had higher maximum  $P_n$  values under AMB ozone conditions. When exposed to 2xAMB ozone, seedlings of clone 3088 demonstrated a distinct reduction in  $P_n$  with increasing temperature at high light intensities (Figures 27c and 27d). Although having greater maximum values, the  $P_n$  response to light and temperature for seedlings of clone 3399 under AMB ozone was very similar to that for seedlings of clone 3088 grown under AMB ozone. In contrast to seedlings of clones 3087 and 3088, there was little difference in the maximum  $P_n$  values between seedlings grown under the AMB and 2xAMB ozone treatments. Unique to seedlings of clone 3399 was a substantial reduction in  $P_n$  at temperatures below approximately 23 °C (Figures 27e and 27f).

Table 26. Summary of ANACOV for mature branch response surface measurements. Highlighted p values indicate significant ( $p=0.05$ ) sources of variation. Coefficients of determination ( $R^2$ ) and standard error of estimate ( $S_{y\cdot}$ ) are measures of model fit and precision, respectively.

Summary of Mature Branch Response Surface ANACOV						
Source	August-September			November		
	DF	$P_a$ ( $p>F$ )	$g_a$ ( $p>F$ )	DF	$P_a$ ( $p>F$ )	$g_a$ ( $p>F$ )
Acid Rain (A)	1	0.987	<b>0.041</b>	1	0.166	0.103
Genotype (G)	2	0.242	0.111	2	0.171	0.931
A x G	2	0.818	0.528	2	0.417	0.277
Error I	5			5		
Ozone (O)	1	0.085	0.236	1	<b>0.009</b>	0.695
A x O	1	0.322	0.563	1	0.147	0.782
G x O	2	0.665	0.290	2	0.059	0.459
A x G x O	2	0.891	0.739	2	0.095	0.869
Error II	4			4		
Age-class (C)	1	<b>0.003</b>	0.269	1	<b>&lt;0.001</b>	0.483
A x C	1	0.359	0.827	1	0.634	0.696
G x C	2	0.992	0.826	2	<b>0.021</b>	0.853
A x G x C	2	0.114	0.752	2	0.160	0.665
Error III	5			5		
A x G x O x C	4	<b>&lt;0.001</b>	<b>&lt;0.001</b>	4	<b>&lt;0.001</b>	<b>&lt;0.001</b>
Lite (L)	1	<b>&lt;0.001</b>	<b>0.012</b>	1	<b>&lt;0.001</b>	<b>&lt;0.001</b>
L <sup>2</sup>	1	<b>&lt;0.001</b>	0.816	1	<b>&lt;0.001</b>	0.599
Temp. (T)	1	<b>&lt;0.001</b>	0.683	1	<b>&lt;0.001</b>	0.198
T <sup>2</sup>	1	<b>&lt;0.001</b>	0.915	1	<b>&lt;0.001</b>	0.475
VPD (V)	1	0.614	<b>&lt;0.001</b>	1	<b>&lt;0.001</b>	<b>&lt;0.001</b>
V <sup>2</sup>	1	0.872	<b>&lt;0.001</b>	1	0.405	<b>0.035</b>
L x T	1	0.224	<b>0.009</b>	1	0.799	0.381
L x V	1	0.146	<b>0.032</b>	1	<b>0.014</b>	<b>0.026</b>
T x V	1	0.966	0.155	1	<b>0.021</b>	0.465
L x T x V	1	0.594	<b>0.026</b>	1	0.053	<b>0.047</b>
Error IV	363			294		
R <sup>2</sup>		0.836	0.705		0.901	0.691
S <sub>y·</sub>		0.738	0.050		0.548	0.052

Table 27. Summary of ANACOV for seedling response surface measurements. Highlighted p values indicate significant ( $p=0.05$ ) sources of variation. Coefficients of determination ( $R^2$ ) and standard error of estimate ( $S_{y\cdot}$ ) are measures of model fit and precision, respectively.

Summary of Seedling Response Surface ANACOV						
Source	August-September			November		
	DF	$P_a$ ( $p>F$ )	$g_a$ ( $p>F$ )	DF	$P_a$ ( $p>F$ )	$g_a$ ( $p>F$ )
Acid Rain (A)	1	0.454	0.778	1	0.440	0.155
Ozone (O)	1	0.098	0.764	2	<b>0.019</b>	0.432
A x O	1	0.345	0.255	2	0.649	0.843
Error I	4			5		
Genotype (G)	2	0.248	<b>0.004</b>	1	<b>0.005</b>	<b>0.004</b>
A x G	2	0.455	<b>0.013</b>	1	<b>&lt;0.001</b>	<b>0.001</b>
O x G	2	0.442	0.700	2	<b>&lt;0.001</b>	0.526
A x O x G	2	0.395	0.115	2	<b>0.042</b>	0.233
Error II	8			4		
Age-class (C)	1	<b>&lt;0.001</b>	<b>0.004</b>	NA	NA	NA
A x C	1	0.438	0.614	NA	NA	NA
O x C	1	0.328	<b>0.012</b>	NA	NA	NA
A x O x C	1	0.585	<b>0.036</b>	NA	NA	NA
Error III	4			NA		
A x O x G x C	4	<b>&lt;0.001</b>	<b>&lt;0.001</b>	NA	NA	NA
Lite (L)	1	0.075	0.724	1	<b>&lt;0.001</b>	0.516
L <sup>2</sup>	1	<b>&lt;0.001</b>	0.997	1	<b>&lt;0.001</b>	0.624
Temp. (T)	1	<b>0.003</b>	0.361	1	<b>&lt;0.001</b>	<b>0.049</b>
T <sup>2</sup>	1	<b>&lt;0.001</b>	0.534	1	<b>&lt;0.001</b>	0.075
VPD (V)	1	0.514	<b>0.001</b>	1	0.132	<b>&lt;0.001</b>
V <sup>2</sup>	1	0.277	<b>0.010</b>	1	0.819	0.205
L x T	1	<b>0.007</b>	0.346	1	0.311	0.405
L x V	1	0.774	0.910	1	0.929	0.376
T x V	1	0.161	0.385	1	0.353	<b>0.025</b>
L x T x V	1	0.260	0.790	1	0.801	0.361
Error IV	354			170		
R <sup>2</sup>		0.891	0.590		0.854	0.691
S <sub>y<math>\cdot</math></sub>		0.694	0.056		0.633	0.052

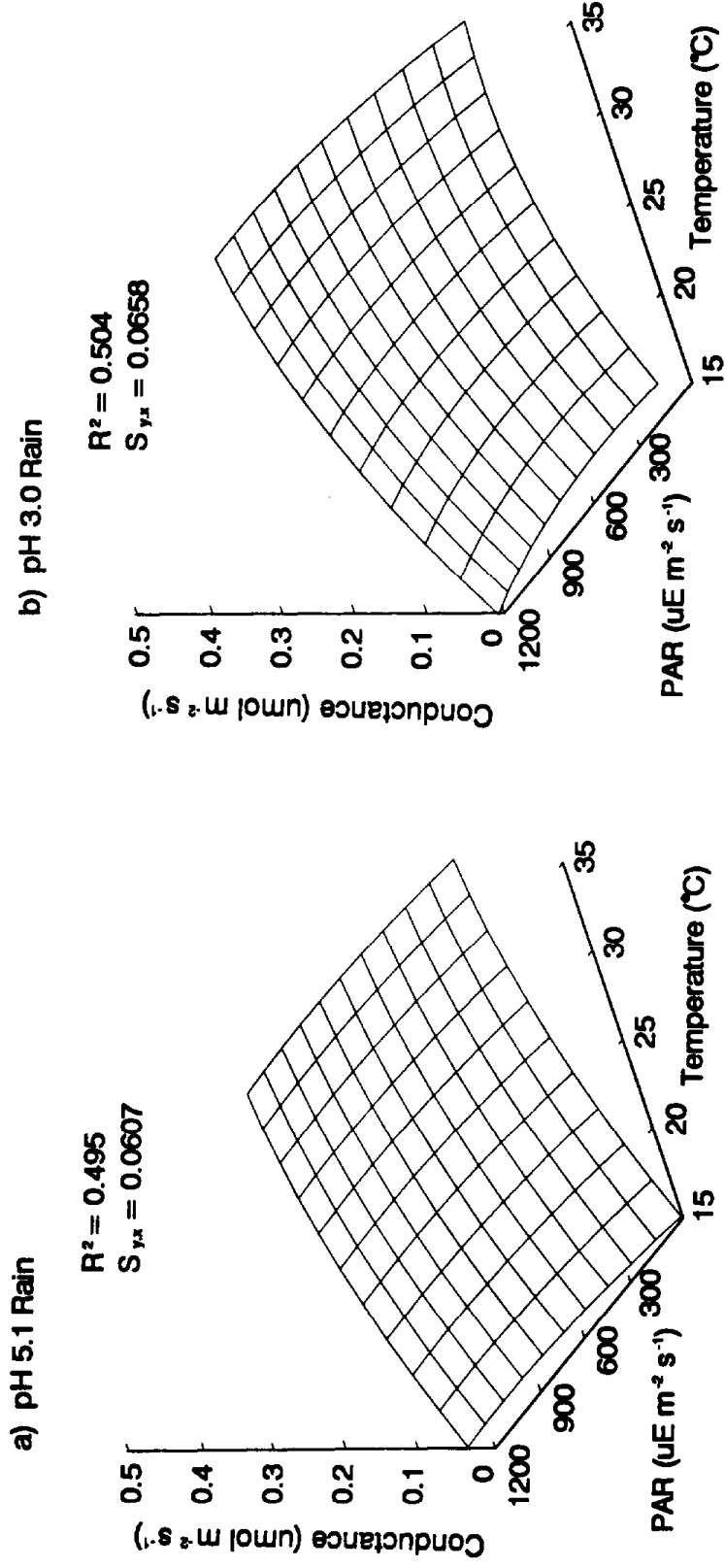


Figure 21. Stomatal conductance light and temperature response surfaces for *Pinus ponderosa* mature branches exposed to a) pH 5.1 rain and b) pH 3.0 rain as measured in August-September. Surfaces presented represent the response at a vapor pressure deficit of 1 kPa.

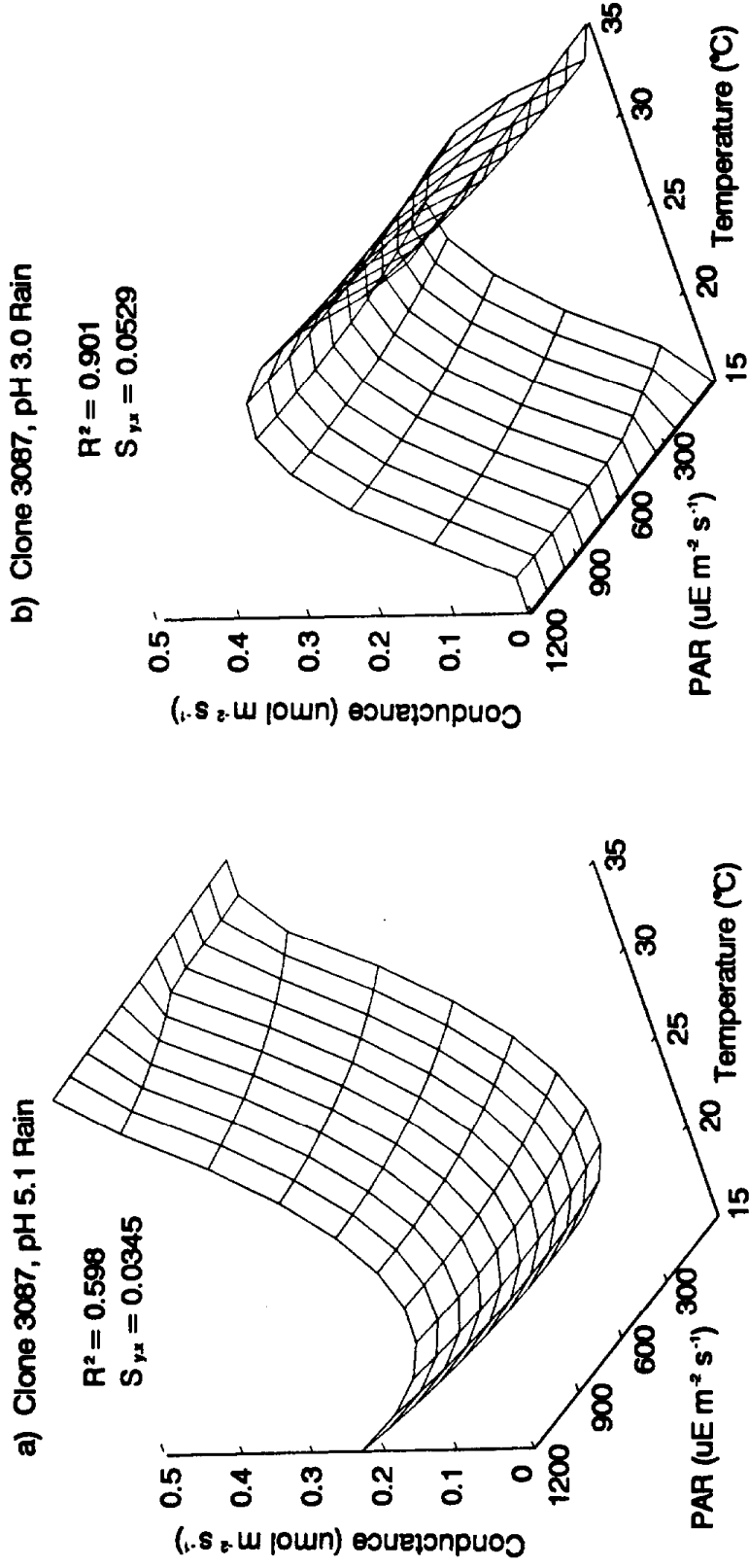


Figure 22a-b. Stomatal Conductance light and temperature response surfaces for *Pinus ponderosa* half-sib seedlings of genotype 3087 exposed to a) pH 5.1 rain and b) pH 3.0 rain as measured in November. Surfaces presented represent the response at a vapor pressure deficit of 1 kPa.

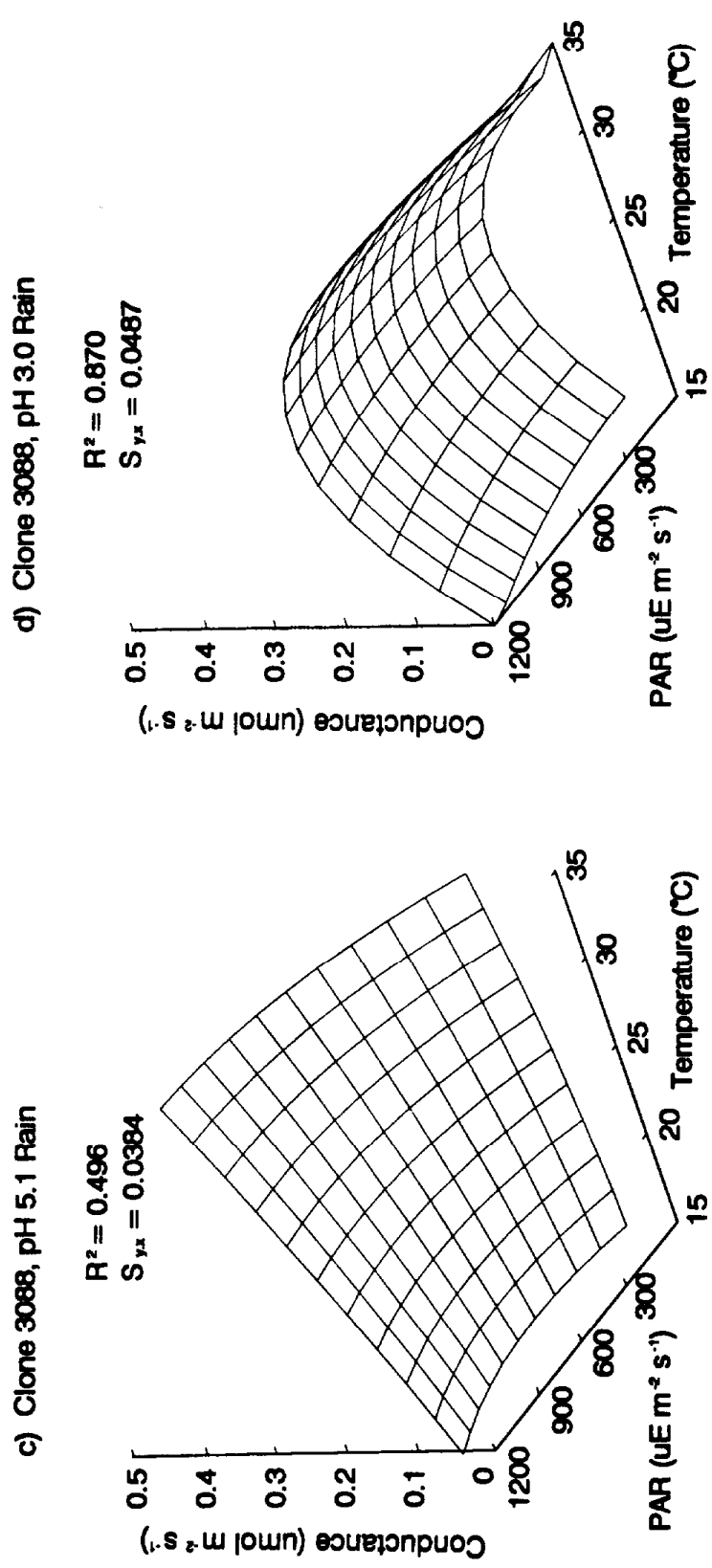


Figure 22c-d. Stomatal Conductance light and temperature response surfaces for *Pinus ponderosa* half-sib seedlings of genotype 3088 exposed to c) pH 5.1 rain and d) pH 3.0 rain as measured in November. Surfaces presented represent the response at a vapor pressure deficit of 1 kPa.

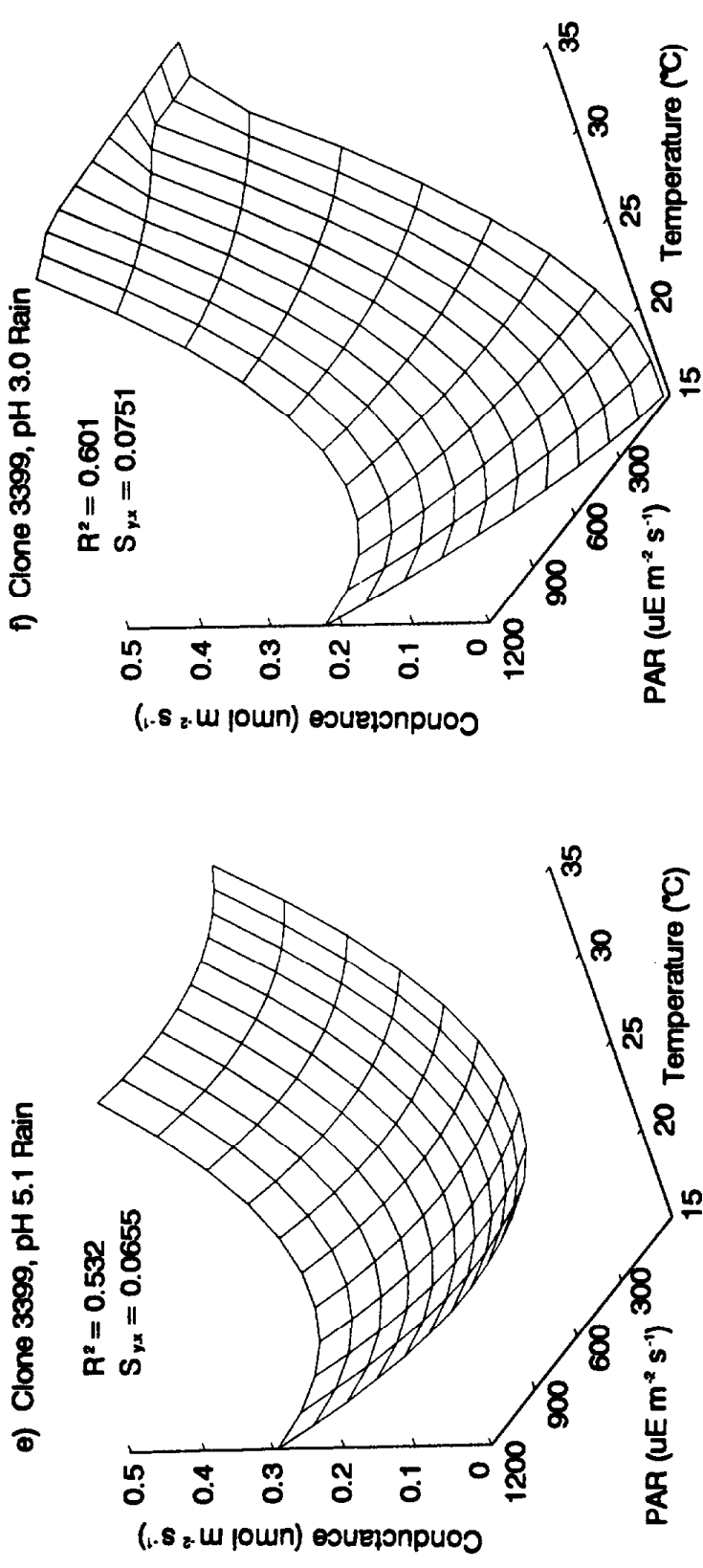


Figure 22e-f. Stomatal Conductance light and temperature response surfaces for *Pinus ponderosa* half-sib seedlings of genotype 3399 exposed to e) pH 5.1 rain and f) pH 3.0 rain as measured in November. Surfaces presented represent the response at a vapor pressure deficit of 1 kPa.

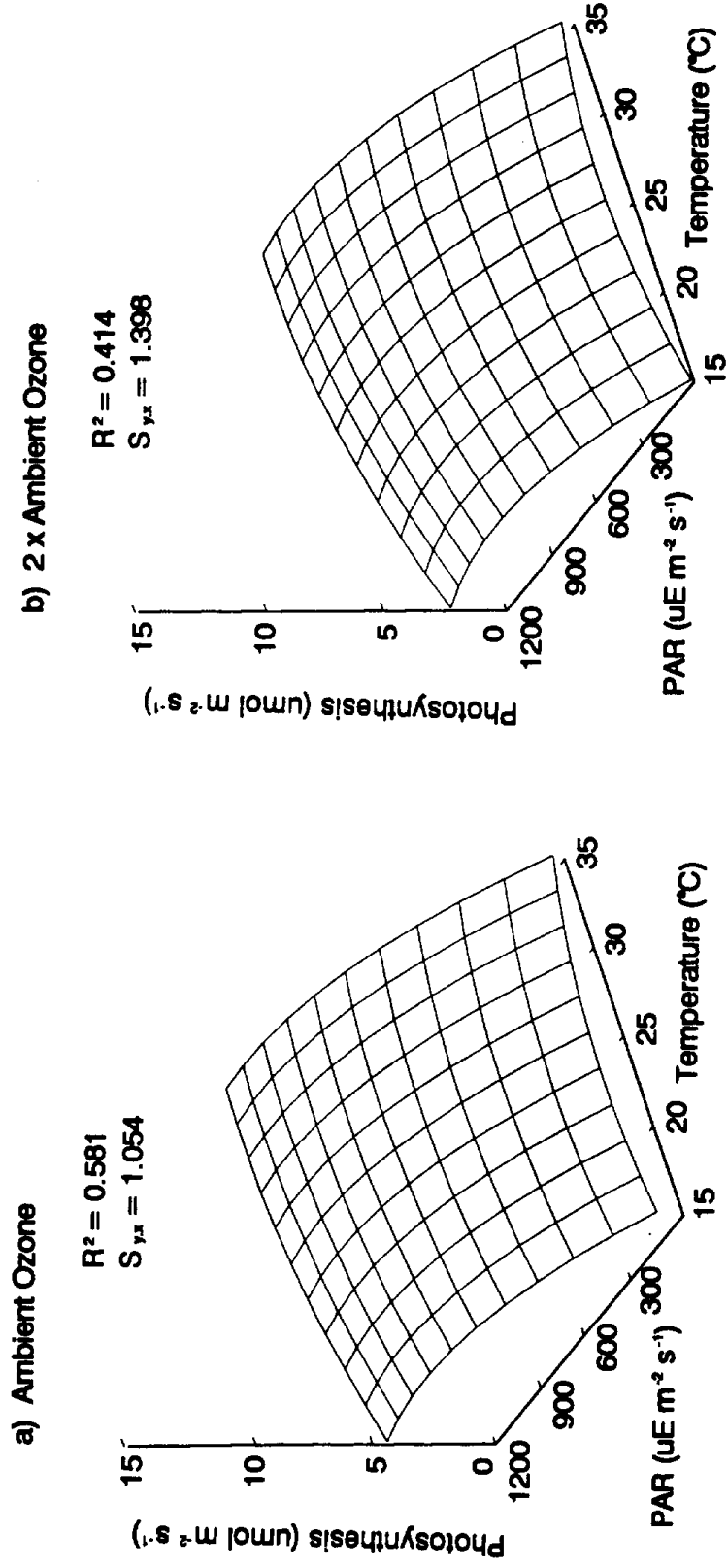
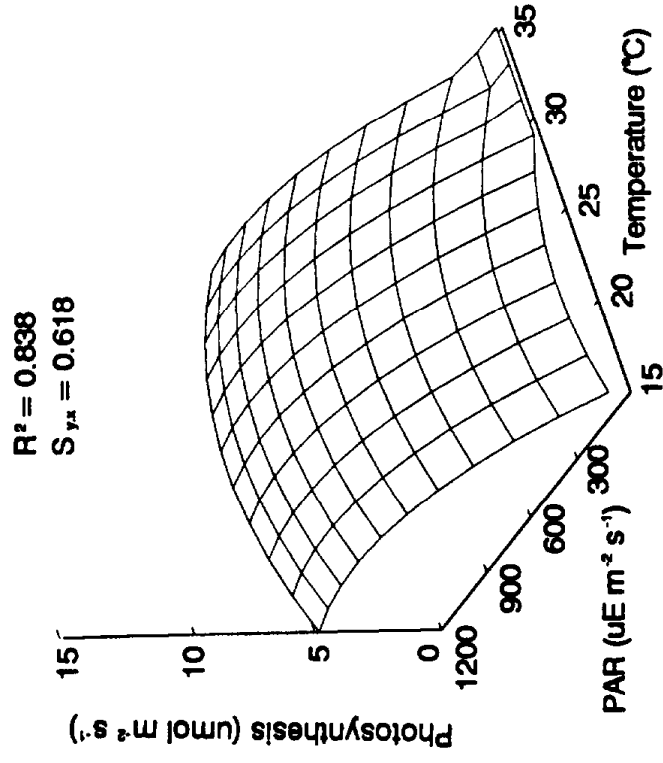


Figure 23. Net photosynthesis light and temperature response surfaces for *Pinus ponderosa* mature branch foliage exposed to a) ambient and b) twice ambient ozone concentrations as measured in August-September. Surfaces presented represent the response at a vapor pressure deficit of 1 kPa.

a) Clone 3087, Ambient Ozone



b) Clone 3087, 2 x Ambient Ozone

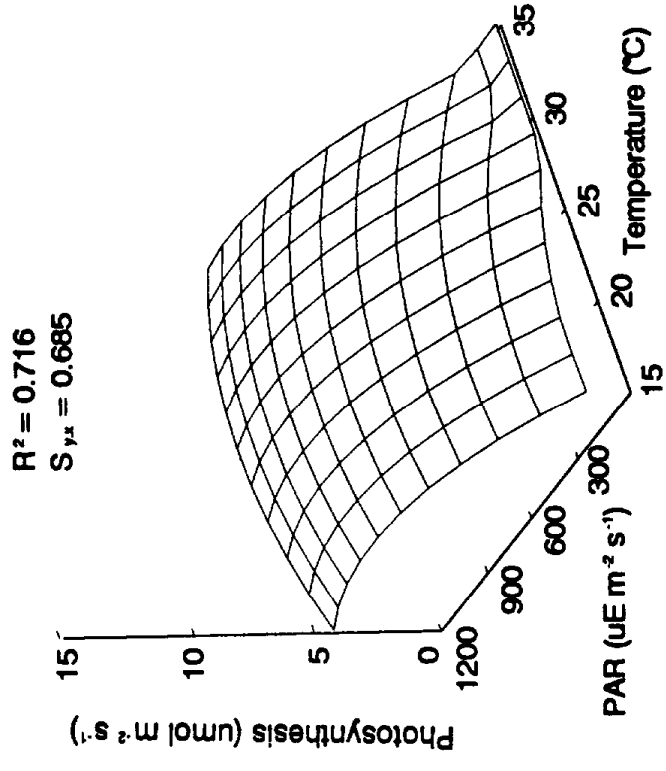
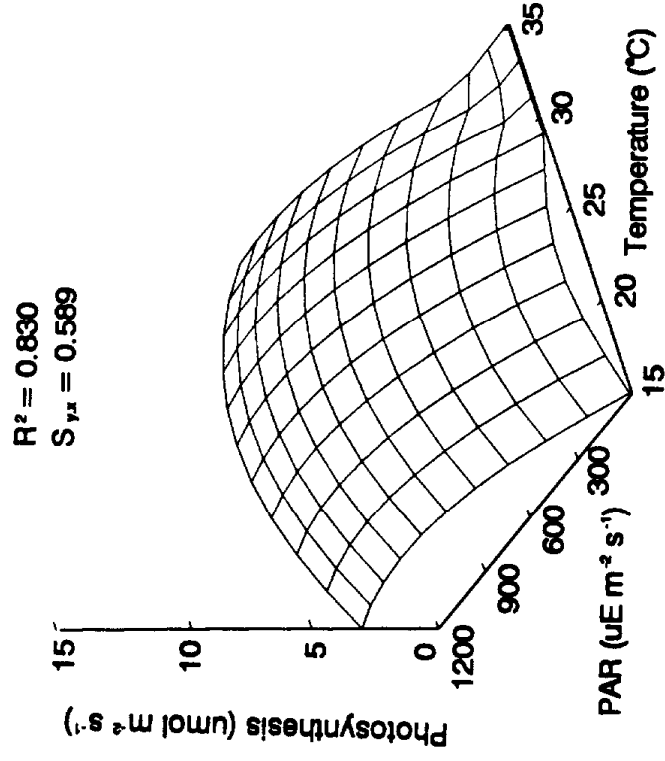


Figure 24a-b. Net photosynthesis light and temperature response surfaces for *Pinus ponderosa* mature branch genotype 3087 exposed to a) ambient and b) twice ambient ozone concentrations as measured in November. Surfaces presented represent the response at a vapor pressure deficit of 1 kPa.

c) Clone 3088, Ambient Ozone



d) Clone 3088, 2 x Ambient Ozone

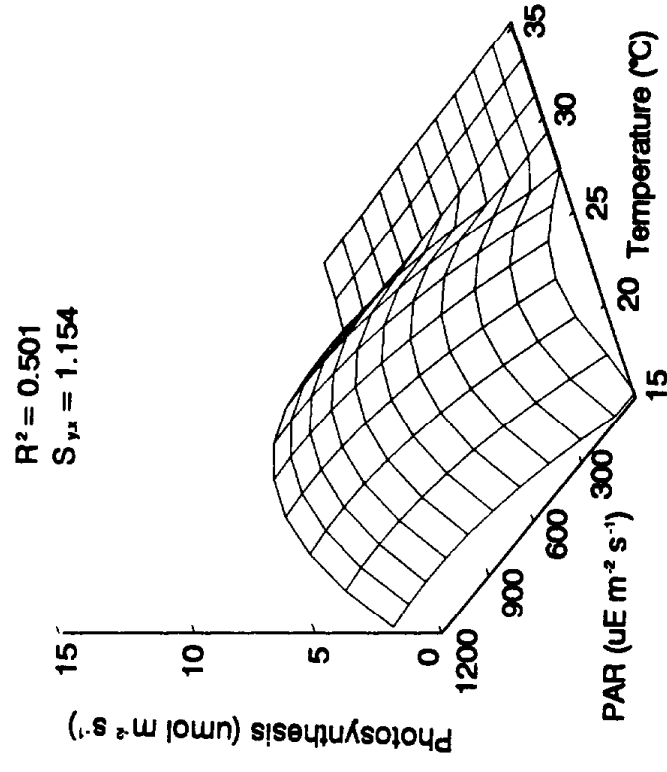
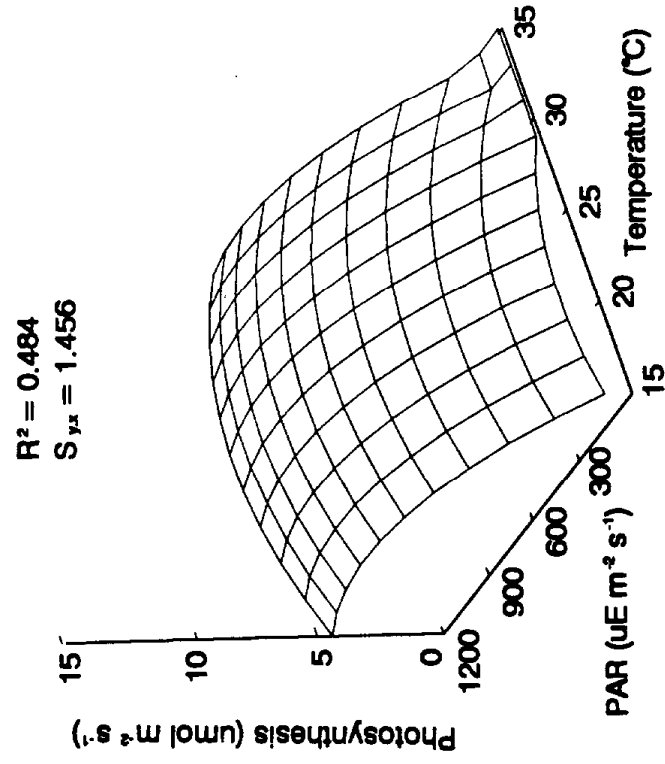


Figure 24c-d. Net photosynthesis light and temperature response surfaces for *Pinus ponderosa* mature branch genotype 3088 exposed to c) ambient and d) twice ambient ozone concentrations as measured in November. Surfaces presented represent the response at a vapor pressure deficit of 1 kPa.

e) Clone 3399, Ambient Ozone



f) Clone 3399, 2 x Ambient Ozone

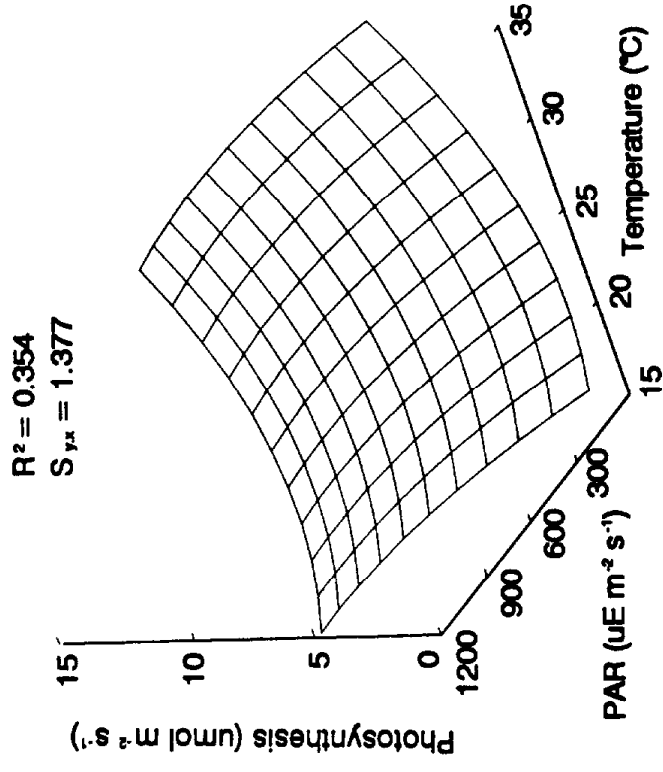


Figure 24e-f. Net photosynthesis light and temperature response surfaces for *Pinus ponderosa* mature branch genotype 3399 exposed to e) ambient and f) twice ambient ozone concentrations as measured in November. Surfaces presented represent the response at a vapor pressure deficit of 1 kPa.

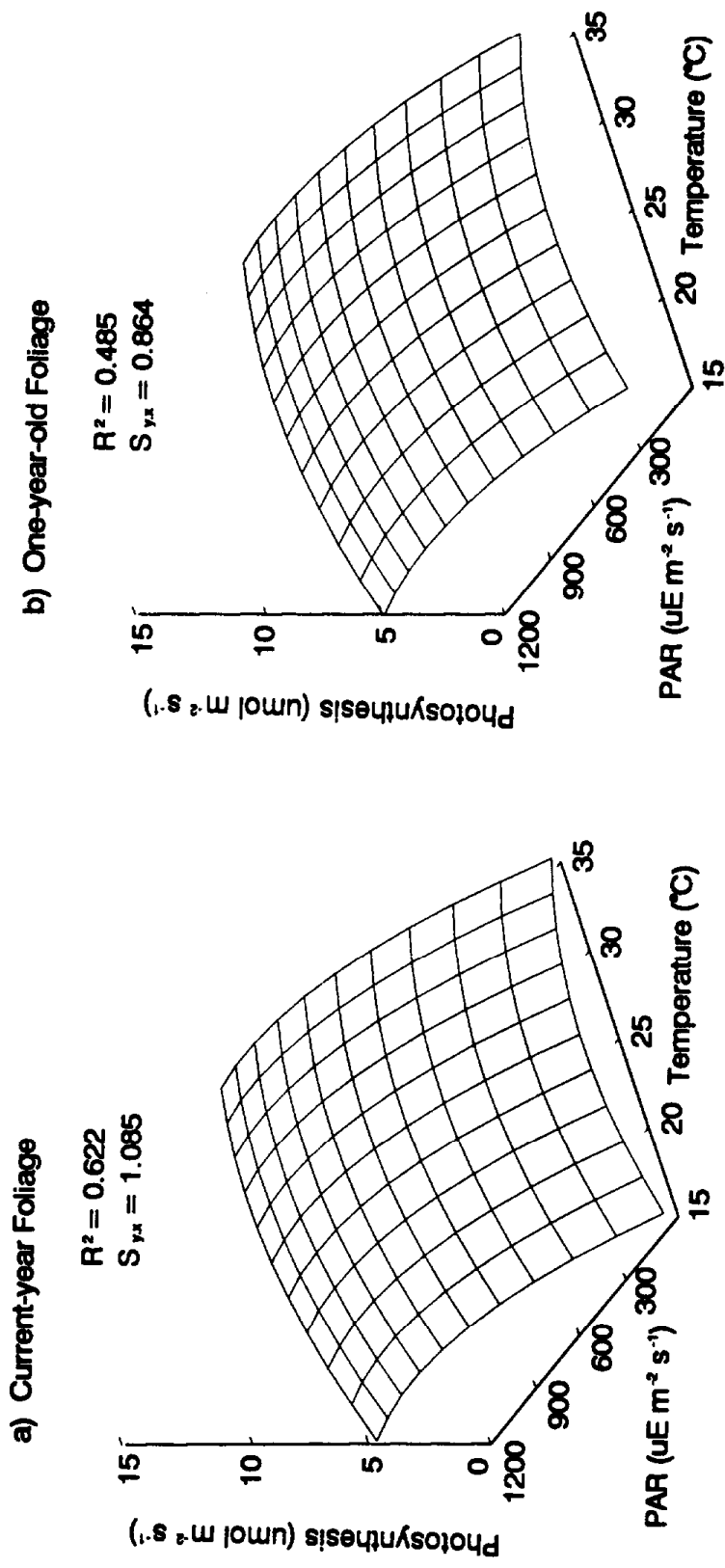
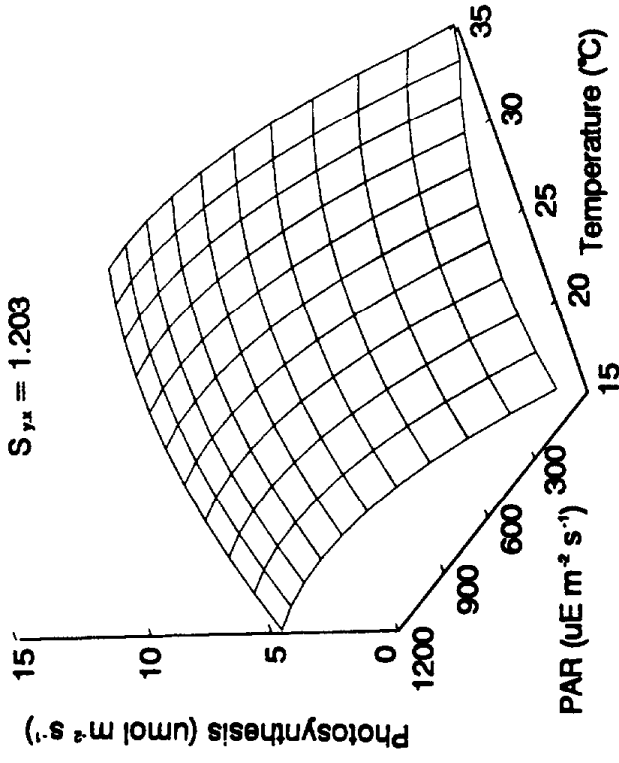


Figure 25. Net photosynthesis light and temperature response surfaces for *Pinus ponderosa* mature branch a) current-year and b) one-year-old foliage as measured in August-September. Surfaces presented represent the response at a vapor pressure deficit of 1 kPa.

a) Current-year Foliage

$$R^2 = 0.537$$

$$S_{yx} = 1.203$$



b) One-year-old Foliage

$$R^2 = 0.161$$

$$S_{yx} = 0.958$$

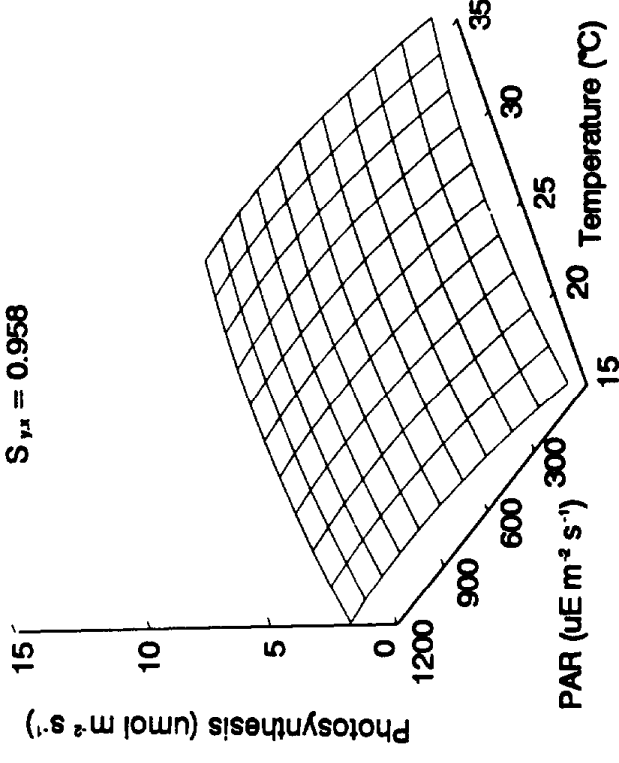
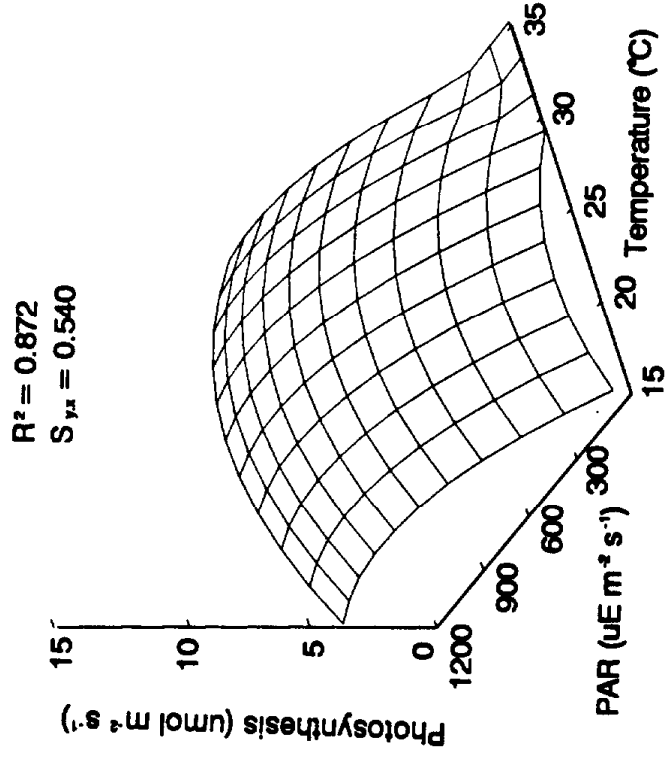


Figure 26. Net photosynthesis light and temperature response surfaces for *Pinus ponderosa* seedling a) current-year and b) one-year-old foliage as measured in August-September. Surfaces presented represent the response at a vapor pressure deficit of 1 kPa.

a) Clone 3087, Ambient Ozone



b) Clone 3087, 2 x Ambient Ozone

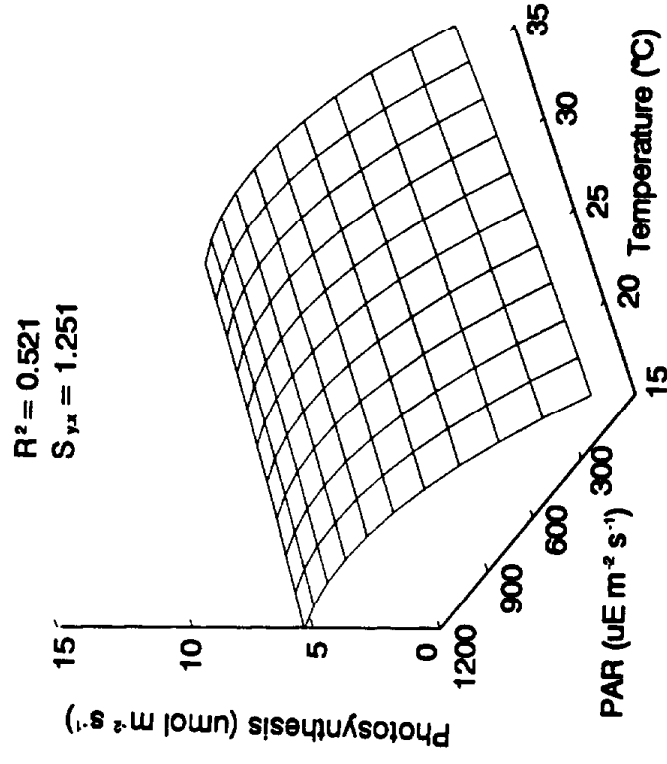
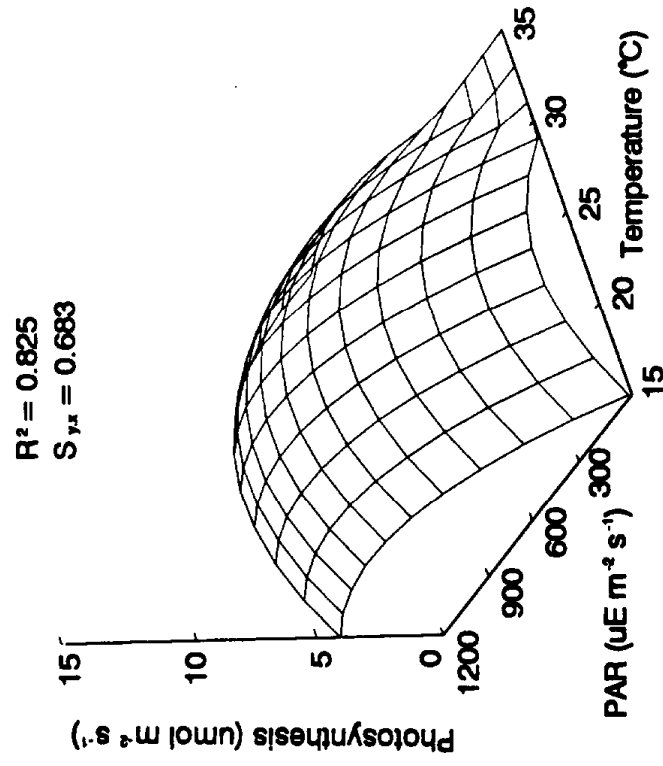


Figure 27a-b. Stomatal Conductance light and temperature response surfaces for *Pinus ponderosa* half-sib seedlings of genotype 3087 exposed to a) pH 5.1 rain and b) pH 3.0 rain as measured in November. Surfaces presented represent the response at a vapor pressure deficit of 1.0 kPa.

c) Clone 3088, Ambient Ozone



d) Clone 3088, 2 x Ambient Ozone

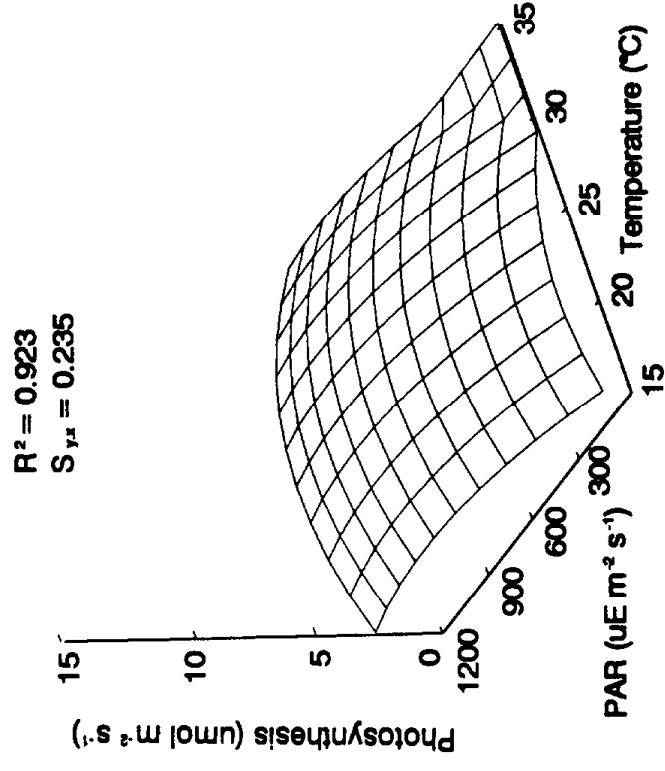
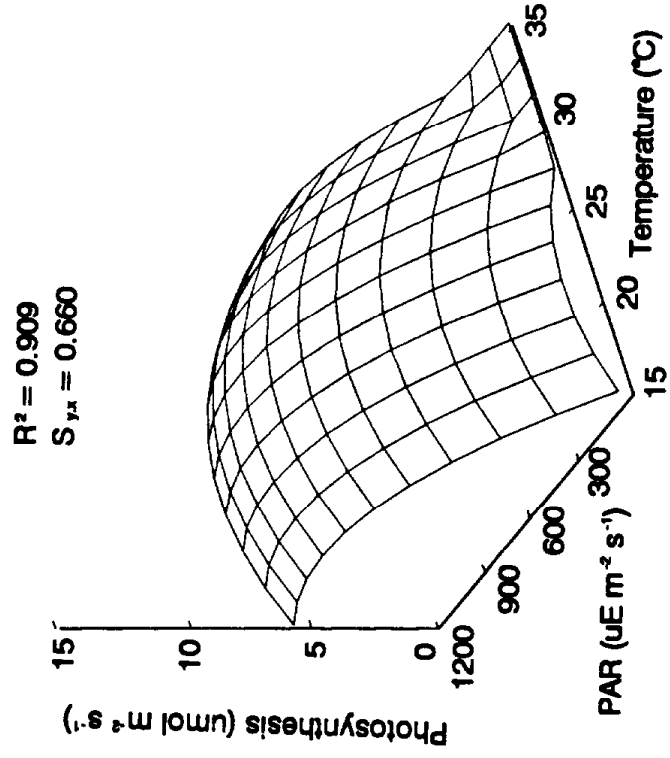


Figure 27c-d. Stomatal Conductance light and temperature response surfaces for *Pinus ponderosa* half-sib seedlings of genotype 3088 exposed to c) pH 5.1 rain and d) pH 3.0 rain as measured in November. Surfaces presented represent the response at a vapor pressure deficit of 1.0 kPa.

e) Clone 3399, Ambient Ozone



f) Clone 3399, 2 x Ambient Ozone

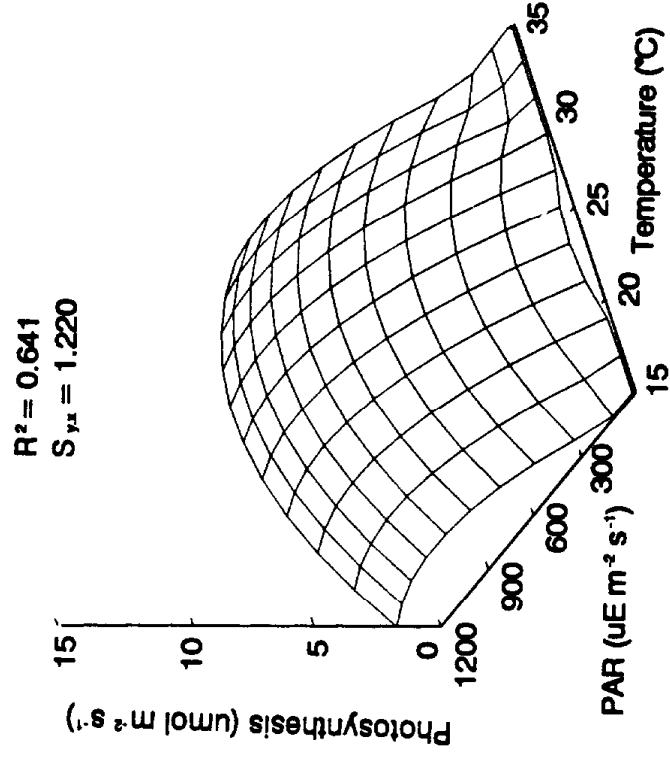


Figure 27e-f. Stomatal Conductance light and temperature response surfaces for *Pinus ponderosa* half-sib seedlings of genotype 3399 exposed to a) pH 5.1 rain and b) pH 3.0 rain as measured in November. Surfaces presented represent the response at a vapor pressure deficit of 1.0 kPa.

## E. Foliar Pigmentation

### 1. Late-Spring

#### a. Mature Branch Pigmentation

##### i. Pigment concentration over all pollutant treatments and genotypes

In May 1992, foliar surface-area concentrations of chlorophyll a (ChlA), chlorophyll b (ChlB) and carotenoids (Car) for one-year-old branch foliage were 18.4, 4.8 and 6.5  $\mu\text{g cm}^{-2}$ , respectively (Table 28). The mean ratio of chlorophyll a to chlorophyll b (ChlA/B) was 3.9 (Table 28).

##### ii. Interactive effect of genotype and ozone (G x O)

Mean ChlA/B values for clones 3087, 3088 and 3399 were 4.01, 4.01 and 3.61, respectively with the ratio for clone 3399 being significantly less than the ratios for the other two genotypes (Table 30). This significant genotypic variation ( $p=0.023$ , Table 29) arose because under CF and AMB ozone, ChlA concentrations for clone 3399 were approximately 35 percent greater than those of clones 3087 and 3088 while the concentration of ChlB was approximately 50 percent greater (Table 30).

The concentrations of ChlA, ChlB and Car varied significantly among clones and the responses to genotype were confounded by ozone treatment (Table 29). For genotypes 3087 and 3088, ChlA, ChlB and CAR did not differ among ozone treatments (Table 30). For genotype 3399, ChlA, ChlB and CAR concentrations were respectively, 42, 47, and 34 percent greater for the CF and AMB treatments than for the 2xAMB treatment (Table 30). Under CF and AMB ozone treatments, the concentrations of pigments were significantly greater for clone 3399 than for either clone 3087 or 3088 (Table 30). Under 2xAMB ozone, there were no differences in pigment concentrations among the three clones (Table 30).

#### b. Seedlings

##### i. Pigment concentration over all pollutant treatments and genotypes

Samples of one-year-old seedling foliage collected in May, 1992 had mean pigment concentrations of 18.5  $\mu\text{g cm}^{-2}$  for ChlA, 5.0  $\mu\text{g cm}^{-2}$  for ChlB and 5.9  $\mu\text{g cm}^{-2}$  for Car (Table 28). Mean ChlA/B was 3.9 (Table 28). For ChlA, Car and ChlA/B, there were no significant ( $p=0.05$ ) effects of acid rain, ozone, genotype or their interactions (Table 31).

ii. Interactive effects of acid rain, genotype and ozone (A x G, A x O x G)

The concentration of ChlB was significantly influenced by the interaction among acid rain and genotype ( $p=0.008$ ) and the interaction among acid rain, ozone and genotype ( $p=0.006$ ) (Table 31). For clones 3088 and 3399, ChlB increased with exposure to increasing acidity. For clone 3087, the highest ChlB concentration occurred in foliage exposed to NAP while the lowest concentration occurred in foliage exposed to simulated rain of pH 5.1 (Table 32).

The three-way interaction among acid rain, ozone and genotype is difficult to interpret as trends in the observed differences are lacking. The interaction is generally due to variation among the three genotypes in the relative ChlB response to CF and AMB ozone treatments under the NAP and pH 5.1 rain treatments (Figure 28). For most genotype x acid rain combinations, ChlB tends to be greater for CF and AMB treatments than for 2xAMB ozone. Exceptions to this trend were evident for genotype 3088 exposed to pH 5.1 rain and for genotype 3087 exposed to NAP. In the first case, ChlB concentrations for 2xAMB ozone tend to be greater than or equal to those for CF and AMB ozone. In the second case, ChlB concentrations for the high ozone treatment tend to be greater than those of the AMB treatment and less than those of the CF treatment.

2. Late-Summer

a. Mature Branch Pigmentation

i. Pigment concentration over all pollutant treatments and genotypes

Mean pigment concentrations measured in current-year foliage of mature branch tissue sampled in September, 1992 ranged from 12.0  $\mu\text{g cm}^{-2}$  for ChlA, 3.8  $\mu\text{g cm}^{-2}$  for ChlB and 3.6  $\mu\text{g cm}^{-2}$  for Car (Table 33). In one-year-old foliage, the mean concentrations were 12.7  $\mu\text{g cm}^{-2}$  for ChlA, 4.9  $\mu\text{g cm}^{-2}$  for ChlB and 4.0  $\mu\text{g cm}^{-2}$  for Car (Table 33). Mean ChlA/B was 3.4 for current-year foliage and 2.7 for one-year-old foliage (Table 33).

ii. Effect of genotype, age-class and genotype x age-class interaction (G, C, G x C)

The concentration of ChlB differed significantly between foliage age-classes for mature branches sampled in September ( $p<0.001$ , Table 34). The mean concentrations were 3.8 and 4.9  $\mu\text{g cm}^{-2}$  for current-year and one-year-old foliage (Table 33).

ChlA/B was significantly greater ( $p<0.001$ , Table 34) for current-year foliage than for one-year-old foliage (Table 35). This corresponds to the significantly greater ChlB concentration observed for one-year-old foliage. The ratio of chlorophyll concentrations also varied significantly among genotypes ( $p=0.043$ , Table 34). Mean ChlA/B for mature branch foliage of clones 3088, 3087 and 3399 was, respectively, 2.7, 3.0 and 3.3 (Table 35).

There was a significant interactive effect of genotype and foliage age-class on ChlA concentrations in mature branch foliage ( $p=0.005$ , Table 34). Trees of clone 3088 had significantly lower ChlA concentrations than trees of clone 3399 (Table 35). For trees of clone 3087, the ChlA concentration was greater in one-year-old foliage than in current-year foliage. For trees of clone 3399, there was no difference in ChlA concentration between age-classes (Table 35).

Carotenoid concentrations of mature branch foliage sampled in September had significant genotype x age-class interaction responses very similar to those observed for ChlA (Tables 34 and 35). Foliage of clone 3088 had the lowest Car concentration and foliage of clone 3399 had the highest Car concentration. For both clones 3088 and 3399, there were no differences in Car concentration between foliage age-classes. The Car concentration of clone 3087 current-year foliage was intermediate to that for current-year foliage of the other clones. The Car concentration of one-year-old foliage was significantly greater than that of current-year foliage for clone 3087 and did not differ significantly from the Car concentration of clone 3399 foliage.

### iii. Effect of acid rain, ozone and acid rain x ozone interaction (A, O, A x O)

There was a significant interactive effect of ozone and acid rain on ChlA concentration (Tables 34 and 36). For all levels of acid rain, ChlA concentration was lowest under 2xAMB ozone exposure. In the pH 5.1 rain treatment, ChlA concentration tended to decline with increasing ozone exposure. In the NAP and pH 3.0 rain treatments, ChlA concentration tended to be less in the CF ozone treatment than in the AMB ozone treatment.

For tissue exposed to pH 5.1 and 3.0, the lowest Car concentrations were observed in foliage receiving 2xAMB ozone (Table 36). Under NAP, Car concentrations were significantly lower in tissues exposed to the CF treatment relative to tissues exposed to the AMB treatment. For tissues exposed to NAP and pH 3.0, the highest Car concentrations were observed in tissues exposed to AMB ozone. For tissues exposed to pH 5.1 rain, the highest Car concentrations occurred under CF conditions. This acid rain x ozone interaction effect on the Car concentration of mature branch foliage was statistically significant ( $p=0.015$ , Table 34).

### iv. Interactive effect of acid rain, genotype and ozone (A x G x O)

There were significant acid rain x genotype x ozone effects on ChlA, ChlB and Car concentrations for mature branch foliage sampled in September ( $p=0.010$ ,  $0.039$ , and  $0.044$ , respectively, Table 32). Among levels of acid rain, the relative ChlA response to the CF and AMB ozone treatments varied for clones 3087 and 3399. For all combinations with the exception of branches receiving NAP and CF, the ChlA concentration was lowest for the 3088 genotype. Under NAP and CF, the ChlA concentration for clone 3088 branches was slightly greater than that for clone 3087 branches (Figure 29). The significant acid rain x genotype x ozone interaction effect on ChlB concentration was due predominantly to among-genotype variation in response to

at the CF and AMB ozone treatments (Figure 30). In general, for all clones and acid rain levels, the lowest concentrations of ChlB occurred under 2xAMB ozone conditions. For foliage of clone 3087 exposed to pH 5.1 rain, there was a tendency for ChlB concentration for the CF treatment to be less than that for the AMB and 2xAMB treatments. For all acid rain treatments, ChlB concentration for clone 3087 differed little between the AMB and 2xAMB treatments. Regardless of acid rain treatment, ChlB concentration of clone 3088 foliage tended to be slightly greater for AMB ozone exposures, relative to CF ozone exposures; and exposure to 2xAMB resulted in the lowest ChlB concentration. For foliage of clone 3399, ChlB concentration declined with an increase in ozone exposure from AMB to 2xAMB levels and at both ozone levels, the ChlB concentration declined with increasing acidity exposure from NAP to pH 3.0. For clone 3399 exposed to the CF ozone treatment, branches exposed to NAP had lower ChlB concentrations than those receiving either pH 3.0 or pH 5.1 rainfall.

The significant acid rain x ozone x genotype interaction effect on Car concentration (Table 32) is very complex and, similar to that for ChlA, is due predominantly to variation among genotypes in the relative Car concentration response to acid rain under CF and AMB ozone conditions (Figure 31).

#### b. Seedling Pigmentation

##### i. Pigment concentration over all pollutant treatments and genotypes

Mean concentrations of pigments measured in current-year seedling foliage sampled in September ranged were  $9.7 \mu\text{g cm}^{-2}$  for ChlA,  $2.9 \mu\text{g cm}^{-2}$  for ChlB,  $3.7 \mu\text{g cm}^{-2}$  for Car (Table 33). The mean concentrations for one-year-old foliage were 7.9, 4.8, and  $6.5 \mu\text{g cm}^{-2}$  for ChlA, ChlB and Car, respectively (Table 33). Mean ChlA/B was 3.6 for current-year foliage and 3.9 for one-year-old foliage (Table 33).

##### ii. Effect of acidic rain (A)

Acid rain had a significant effect on ChlB concentrations in seedling foliage sampled in September ( $p=0.018$ , Table 37). The concentration of ChlB was significantly lower for tissues exposed to pH 3.0 rain ( $2.61 \mu\text{g cm}^{-2}$ ) than for tissues exposed to either NAP ( $3.1 \mu\text{g cm}^{-2}$ ) or pH 5.1 rain ( $3.2 \mu\text{g cm}^{-2}$ ) (Table 38).

##### iii. Effect of foliage age-class (C)

Chlorophyll a concentration differed significantly between foliage age-classes ( $p<0.001$ , Table 37). Mean ChlA for current-year,  $9.6 \mu\text{g cm}^{-2}$ , was greater than that for one-year-old foliage,  $7.6 \mu\text{g cm}^{-2}$  (Table 38). Mean ChlA/B was also significantly greater for current-year foliage (3.67) than for one-year-old foliage (2.52) of seedlings sampled in September ( $p<0.001$ , Tables 37 and 39). In contrast to mature branches, the age-class difference was related to age-class differences in ChlA rather than differences in ChlB.

iv. Effect of ozone, genotype and ozone x genotype interaction (O, G, O x G)

Chlorophyll a concentration varied significantly in response to ozone ( $p < 0.001$ , Table 37). Tissues exposed to 2xAMB ozone had significantly lower ChlA concentrations ( $6.9 \mu\text{g cm}^{-2}$ ) than tissues exposed to either CF or AMB ozone ( $9.5 \mu\text{g cm}^{-2}$  for both) (Table 40).

ChlA/B also varied in response to genotype ( $p = 0.030$ , Table 37). The ratio was greater for seedlings of clone 3087 (3.29) than for seedlings of clone 3088 (2.85). The ratio for seedlings of clone 3399 (3.15) was intermediate and did not differ significantly from ChlA/B values for the other two genotypes (Table 40).

There was also a significant ozone x genotype interaction effect on the concentration of ChlB in seedling foliage (Tables 37 and 40). This interaction arose from differences in the relative responses to CF and AMB ozone treatments among genotypes. Under 2xAMB ozone, there was no difference in the concentration of ChlB among seedlings of the three genotypes. For clone 3087, ChlB concentration was slightly lower (insignificant) in the AMB treatment relative to the CF treatment. For seedlings of clone 3088, there was virtually no difference in ChlB concentrations between the CF and AMB treatments. For clone 3399, the concentration of ChlB was significantly greater in the AMB treatment relative to the CF treatment.

v. Interactive effect of ozone, acid rain and foliage age-class (A x O x C)

Carotenoid concentrations of seedling foliage sampled in September varied significantly among ozone treatments and this response to ozone was confounded by the acid rain and foliage age-class (Table 37 and Figure 32). In general there were lower Car concentrations in foliage exposed to 2xAMB ozone relative to concentrations in foliage exposed to the CF and AMB treatments. When exposed to NAP, the reduction under 2xAMB ozone was expressed in the current-year foliage. When exposed to pH 5.1 rainfall, reductions in Car concentration in response to 2xAMB ozone occurred for both current-year and one-year-old foliage. When exposed to pH 3.0 rainfall, reductions in Car in response to 2xAMB ozone were evident for the older foliage age-class only.

Table 28. Foliar concentrations of chlorophyll and carotenoid pigments for seedling and mature branch one-year-old foliage of *Pinus ponderosa* sampled in May, 1992. Values are means and standard errors of the means calculated over all genotypes and pollutant exposure treatments.

Parameter	Pigment Concentration ( $\mu\text{g cm}^{-2}$ )			ChlA/B Ratio
	ChlA	ChlB	CAR	
Mature Branch One-year-old Foliage				
mean	18.41	4.82	6.51	3.90
s.e.	0.58	0.19	0.18	0.04
Seedling One-year-old Foliage				
mean	18.47	5.03	5.86	3.87
s.e.	0.60	0.19	0.20	0.09

Table 29. Summary of May 1992 mature branch foliar pigment ANOVA.

Mature Branch Pigment ANOVA May 1992									
Source	DF	Chlorophyll a		Chlorophyll b		Carotenoids		Chlorophyll a:b Ratio	
		F	P>F	F	P>F	F	P>F	F	P>F
Acid Rain (A)	2	0.15	0.861	0.21	0.817	0.30	0.742	0.22	0.804
Genotype (G)	2	8.68	<b>0.002</b>	9.37	<b>0.002</b>	6.13	<b>0.009</b>	4.67	<b>0.023</b>
A x G	4	2.18	0.112	1.66	0.204	2.37	0.092	0.33	0.850
Ozone (O)	2	2.84	0.085	2.42	0.117	1.79	0.195	0.13	0.883
A x O	4	0.30	0.876	0.33	0.855	0.58	0.681	0.30	0.874
G x O	4	5.85	<b>0.003</b>	5.84	<b>0.003</b>	5.48	<b>0.005</b>	0.39	0.816
A x G x O	8	0.42	0.894	0.73	0.662	0.30	0.956	1.16	0.372

Table 30. Foliar pigment concentration by genotype and ozone treatment for one-year-old foliage of mature branches sampled in May, 1992. For each pigment, genotype  $\times$  ozone mean values accompanied by a common letter do not differ at the  $p=0.05$  level of significance.

Foliar Pigment Concentration ( $\mu\text{g cm}^{-2}$ ) One-year-old Mature Branch Foliage May, 1992				
Genotype	Ozone Treatment			Overall
	CF	AMB	2xAMB	
Chlorophyll a				
3087	17.81b	16.91b	17.27b	17.33b
3088	14.78b	14.74b	16.45b	15.32b
3399	24.49a	24.73a	17.15b	22.12a
Chlorophyll b				
3087	4.43b	4.22b	4.47b	4.37b
3088	3.72b	3.77b	4.21b	3.90b
3399	6.99a	6.87a	4.67b	6.17a
Carotenoids				
3087	6.57b	6.15b	6.10b	6.27b
3088	5.29b	5.28b	6.15b	5.57b
3399	8.11a	8.27a	6.12b	7.50a
Chlorophyll a:b Ratio				
3087	4.06a	4.06a	3.92a	4.01a
3088	4.05a	4.01a	3.97a	4.01a
3399	3.58b	3.62b	3.67b	3.61b

Table 31. Summary of May 1992 seedling foliar pigment ANOVA.

Seedling Pigment ANOVA May 1992									
Source	DF	Chlorophyll a		Chlorophyll b		Carotenoids		Chlorophyll a:b Ratio	
		F	P>F	F	P>F	F	P>F	F	P>F
Acid Rain (A)	2	0.29	0.750	0.44	0.646	0.38	0.687	0.83	0.445
Ozone (O)	2	2.50	0.101	2.10	0.143	2.14	0.138	1.09	0.351
A x O	4	1.14	0.359	1.01	0.416	2.12	0.106	1.15	0.335
Genotype (G)	2	2.50	0.092	2.41	0.100	1.45	0.244	1.04	0.359
A x G	4	2.25	0.075	3.81	<b>0.008</b>	2.04	0.101	2.53	0.051
O x G	4	0.94	0.446	1.64	0.177	0.66	0.622	0.19	0.940
A x O x G	8	2.05	0.058	3.07	<b>0.006</b>	1.99	0.066	2.10	0.053

Table 32. Foliar concentration of Chlorophyll b for seedlings measured in May, 1992 by acid rain and genotype. Values followed by a common letter do not differ at the  $p=0.05$  level of significance.

Chlorophyll B Concentration ( $\mu\text{g cm}^{-2}$ ) Seedling Foliage May, 1992			
Genotype	Acid Rain Treatment		
	NAP	pH 5.1	pH 3.0
3087	5.66a	3.58b	4.40ab
3088	4.93ab	5.19ab	5.53a
3399	4.56ab	5.05ab	5.74a

Table 33. Foliar concentrations of chlorophyll and carotenoid pigments for seedlings and mature branches of *Pinus ponderosa* sampled in September, 1992. Values are means and standard errors of the means calculated over all genotypes and pollutant exposure treatments.

Parameter	Pigment Concentration ( $\mu\text{g cm}^{-2}$ )			ChlA/B Ratio
	ChlA	ChlB	CAR	
Mature Branch Current-year Foliage				
mean	11.96	3.83	3.59	3.36
s.e.	0.49	0.22	0.12	0.11
Mature Branch One-year-old Foliage				
mean	12.67	4.93	3.99	2.66
s.e.	0.51	0.25	0.14	0.06
Seedling Current-year Foliage				
mean	9.66	2.91	3.70	3.61
s.e.	0.33	0.12	0.12	0.11
Seedling One-year-old Foliage				
mean	7.86	4.82	6.51	3.90
s.e.	0.25	0.11	0.08	0.05

Table 34. Summary of September 1992 mature branch foliar pigment ANOVA

Mature Branch Pigment ANOVA September 1992									
Source	DF	Chlorophyll a		Chlorophyll b		Carotenoids		Chlorophyll a:b Ratio	
		F	P>F	F	P>F	F	P>F	F	P>F
Acid Rain (A)	2	0.67	0.519	0.57	0.570	0.63	0.539	0.20	0.817
Genotype (G)	2	3.95	<b>0.026</b>	1.06	0.355	1.86	0.167	3.38	<b>0.043</b>
A x G	4	0.37	0.831	0.91	0.468	0.21	0.932	1.58	0.197
Ozone (O)	2	11.49	<b>&lt;0.001</b>	5.29	<b>0.009</b>	6.07	<b>0.005</b>	0.40	0.673
A x O	4	4.70	<b>0.003</b>	1.28	0.294	3.46	<b>0.015</b>	0.89	0.479
G x O	4	2.20	0.084	1.76	0.154	1.19	0.329	1.30	0.284
A x G x O	8	2.93	<b>0.010</b>	2.28	<b>0.039</b>	2.21	<b>0.044</b>	1.38	0.232
Age-class (C)	1	1.28	0.264	14.62	<b>&lt;0.001</b>	6.71	<b>0.013</b>	26.86	<b>&lt;0.001</b>
A x C	2	0.70	0.501	0.84	0.439	0.53	0.592	0.45	0.642
G x C	2	6.04	<b>0.005</b>	2.62	0.084	4.03	<b>0.025</b>	2.13	0.130
A x G x C	4	0.89	0.475	0.29	0.880	0.60	0.662	0.92	0.460
A x G x O x C	18	0.98	0.502	1.61	0.099	0.92	0.563	1.17	0.325

Table 35. Foliar concentrations of Chlorophyll a and Carotenoids in mature branch foliage measured in September, 1992 by genotype and foliage age-class. For each parameter, genotype x age-class means, genotype means or age-class means followed by a common letter do not differ at the p=0.05 level of significance.

Mature Branch Foliar Pigment Concentration ( $\mu\text{g cm}^{-2}$ ) September, 1992			
Genotype	1992 Foliage	1991 Foliage	Overall
Chlorophyll a			
3087	11.41b	14.09a	12.54ab
3088	9.51c	9.56c	9.88b
3399	15.12a	14.00a	14.52a
overall	11.96a	12.67a	
Chlorophyll b			
3087	3.66b	5.38a	4.52a
3088	3.26b	4.39ab	3.83a
3399	4.55ab	5.02a	4.79a
overall	3.83b	4.39a	
Carotenoids			
3087	3.49b	4.51a	3.94a
3088	3.16b	3.37b	3.32a
3399	4.17a	4.16a	4.11a
overall	3.59b	3.99a	
Chlorophyll a:b			
3087	3.35a	2.71bc	3.03ab
3088	3.03b	2.47c	2.74b
3399	3.71a	2.81bc	3.26a
overall	3.36a	2.66b	

Table 36. Foliar pigment concentration by acid rain and ozone treatment for foliage of mature branches sampled in September, 1992. For each pigment, values accompanied by a common letter do not differ at the  $\alpha=0.05$  level of significance.

Chlorophyll A and Carotenoid Concentration ( $\mu\text{g cm}^{-2}$ ) Mature Branch Foliage September, 1992			
Acid Rain	Ozone Treatment		
	CF	AMB	2xAMB
Chlorophyll a			
NAP	11.07bc	14.92a	11.65bc
pH 5.1	15.09a	13.33ab	11.03bc
pH 3.0	11.10bc	12.41a	9.87c
Carotenoids			
NAP	3.35b	4.49a	3.73ab
pH 5.1	4.56a	4.11ab	3.52b
pH 3.0	3.47b	3.89ab	3.20b

Table 37. Summary of September 1992 seedling foliar pigment ANOVA.

Seedling Pigment ANOVA September 1992									
Source	DF	Chlorophyll a		Chlorophyll b		Carotenoids		Chlorophyll a:b Ratio	
		F	P>F	F	P>F	F	P>F	F	P>F
Acid Rain (A)	2	0.47	0.629	4.16	<b>0.018</b>	0.14	0.870	2.83	<b>0.063</b>
Ozone (O)	2	9.77	<b>&lt;0.001</b>	8.21	<b>&lt;0.001</b>	4.09	<b>0.019</b>	0.23	0.792
A x O	4	0.28	0.893	0.52	0.724	0.91	0.463	0.81	0.520
Genotype (G)	2	0.60	0.551	1.61	0.204	0.13	0.881	3.60	<b>0.030</b>
A x G	4	0.73	0.572	0.75	0.559	1.64	0.167	0.50	0.733
O x G	4	2.04	0.092	2.78	<b>0.029</b>	1.53	0.198	1.28	0.280
A x O x G	8	0.78	0.625	0.61	0.768	0.81	0.592	1.19	0.311
Age-class (C)	1	22.46	<b>&lt;0.001</b>	2.80	0.097	0.63	0.430	74.47	<b>&lt;0.001</b>
A x C	2	0.31	0.733	0.39	0.679	0.92	0.399	0.84	0.435
O x C	2	0.06	0.942	0.15	0.860	0.62	0.541	0.73	0.483
A x O x C	4	2.02	<b>0.095</b>	0.88	0.479	2.51	<b>0.045</b>	0.51	0.731
A x O x G x C	18	0.69	0.819	0.55	0.926	0.91	0.569	0.78	0.716

Table 38. Foliar concentrations of Chlorophyll a, chlorophyll b and carotenoids for current-year and one-year-old seedling foliage measured in September. For each parameter, foliage age-class means followed by a common letter do not differ at the p=0.05 level of significance.

Seedling Foliar Pigment Concentration ( $\mu\text{g cm}^{-2}$ ) September, 1992		
Pigment	1992 Foliage	1991 Foliage
Chlorophyll a	9.60a	7.64b
Chlorophyll b	2.86a	3.14a
Carotenoids	3.69a	3.60a
Chlorophyll a:b Ratio	3.67a	2.52b

Table 39. September, 1992, concentrations of Chlorophyll a, chlorophyll b and carotenoids for seedling foliage exposed to no acidic rain (NAP), pH 5.1 acidic rain (pH 5.1) or pH 3.0 acidic rain (pH 3.0). For each parameter, foliage age-class means followed by a common letter do not differ at the p=0.05 level of significance.

Seedling Foliar Pigment Concentration ( $\mu\text{g cm}^{-2}$ ) September, 1992			
Pigment	NAP	pH 5.1	pH 3.0
Chlorophyll a	8.95a	8.59a	8.35a
Chlorophyll b	3.13a	3.25a	2.61b
Carotenoids	3.70a	3.57a	3.68a
Chlorophyll a:b Ratio	3.10a	2.84a	3.35a

Table 40. Foliar concentrations of Chlorophyll a, Chlorophyll b and Carotenoids in seedling foliage measured in September, 1992 by ozone treatment and genotype. For each parameter, ozone x genotype means, ozone means or genotype means followed by a common letter do not differ at the p=0.05 level of significance.

Seedling Branch Foliar Pigment Concentration ( $\mu\text{g cm}^{-2}$ ) September, 1992				
Genotype	Ozone Treatment			Overall
	Charcoal Filtered	Ambient	Twice Ambient	
Chlorophyll a				
3087	9.74ab	8.55abc	6.60c	8.27a
3088	10.17a	9.63ab	6.57c	8.82a
3399	8.53abc	10.38a	7.46bc	8.79a
overall	9.47a	9.52a	6.88b	
Chlorophyll b				
3087	3.22ab	2.90ab	2.28c	2.79a
3088	3.46ab	3.51ab	2.55b	3.18a
3399	2.67b	3.82a	2.56b	3.02a
overall	3.12a	3.41a	2.46a	
Carotenoids				
3087	4.15a	3.57ab	3.27ab	3.66a
3088	4.03ab	3.85ab	3.22b	3.71a
3399	3.53ab	3.88ab	3.35ab	3.59a
overall	3.90a	3.76a	3.28a	
Chlorophyll a:b				
3087	3.18a	3.32a	3.44a	3.29a
3088	3.04a	2.83a	2.67a	2.85a
3399	3.42a	3.02a	3.01a	3.15a
overall	3.19a	3.06a	3.05a	

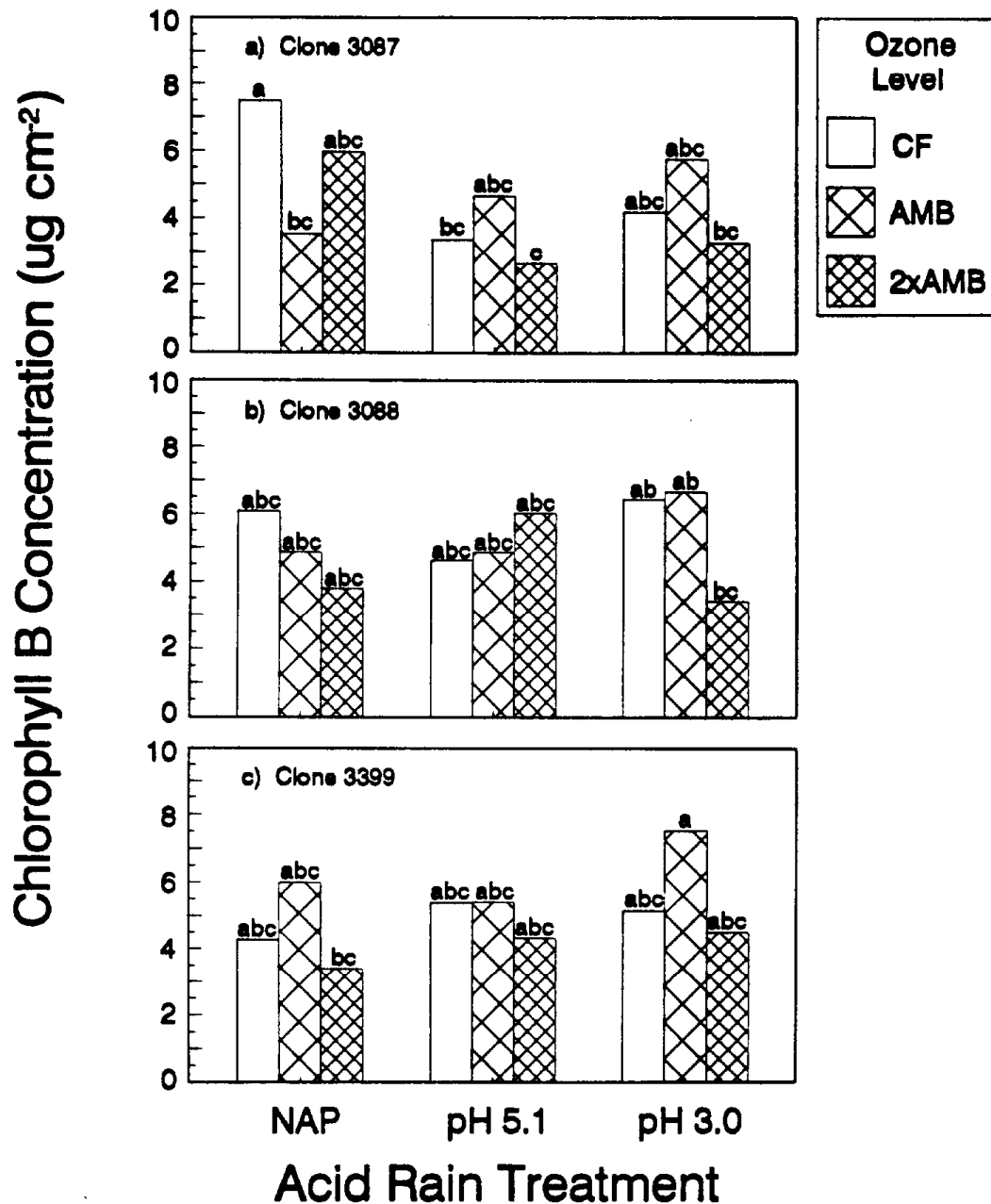


Figure 28. Late-spring chlorophyll b concentration of one-year-old foliage of *Pinus ponderosa* seedlings exposed to no acid rain (NAP), pH 5.1 rain or pH 3.0 rain and charcoal-filtered air (CF), ambient ozone (AMB) or twice ambient ozone (2xAMB) for half-sib genotypes a) 3087, b) 3088 and c) 3399. Among figures a-c, bars denoted by a common letter do not differ at the  $p=0.05$  level of significance.

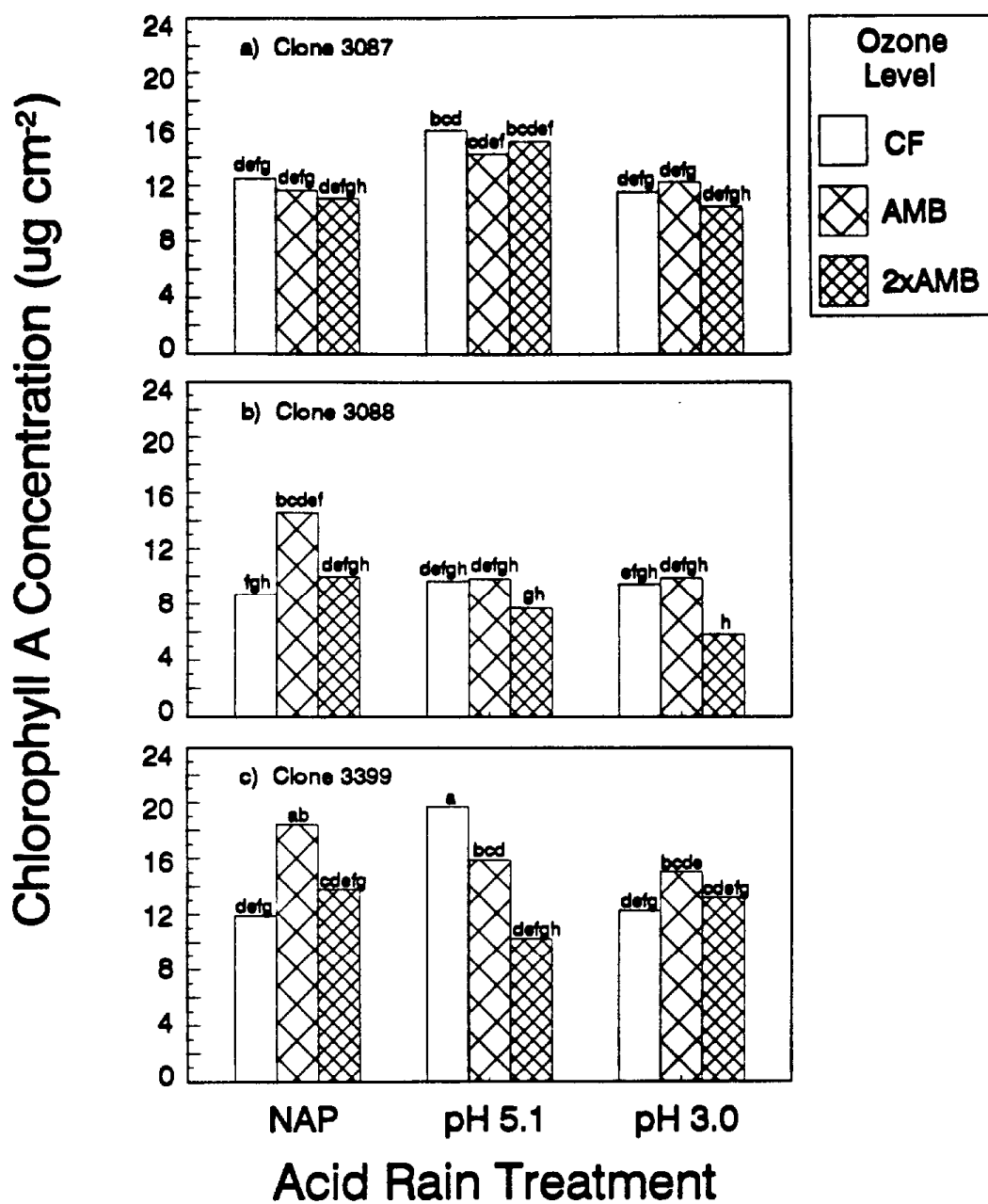


Figure 29. Late-summer foliar chlorophyll a concentration of *Pinus ponderosa* mature branches exposed to no acid rain (NAP), pH 5.1 rain or pH 3.0 rain and charcoal-filtered air (CF), ambient ozone (AMB) or twice ambient ozone (2xAMB) for genotypes a) 3087, b) 3088 and c) 3399. Among figures a-c, bars denoted by a common letter do not differ at the  $p=0.05$  level of significance.

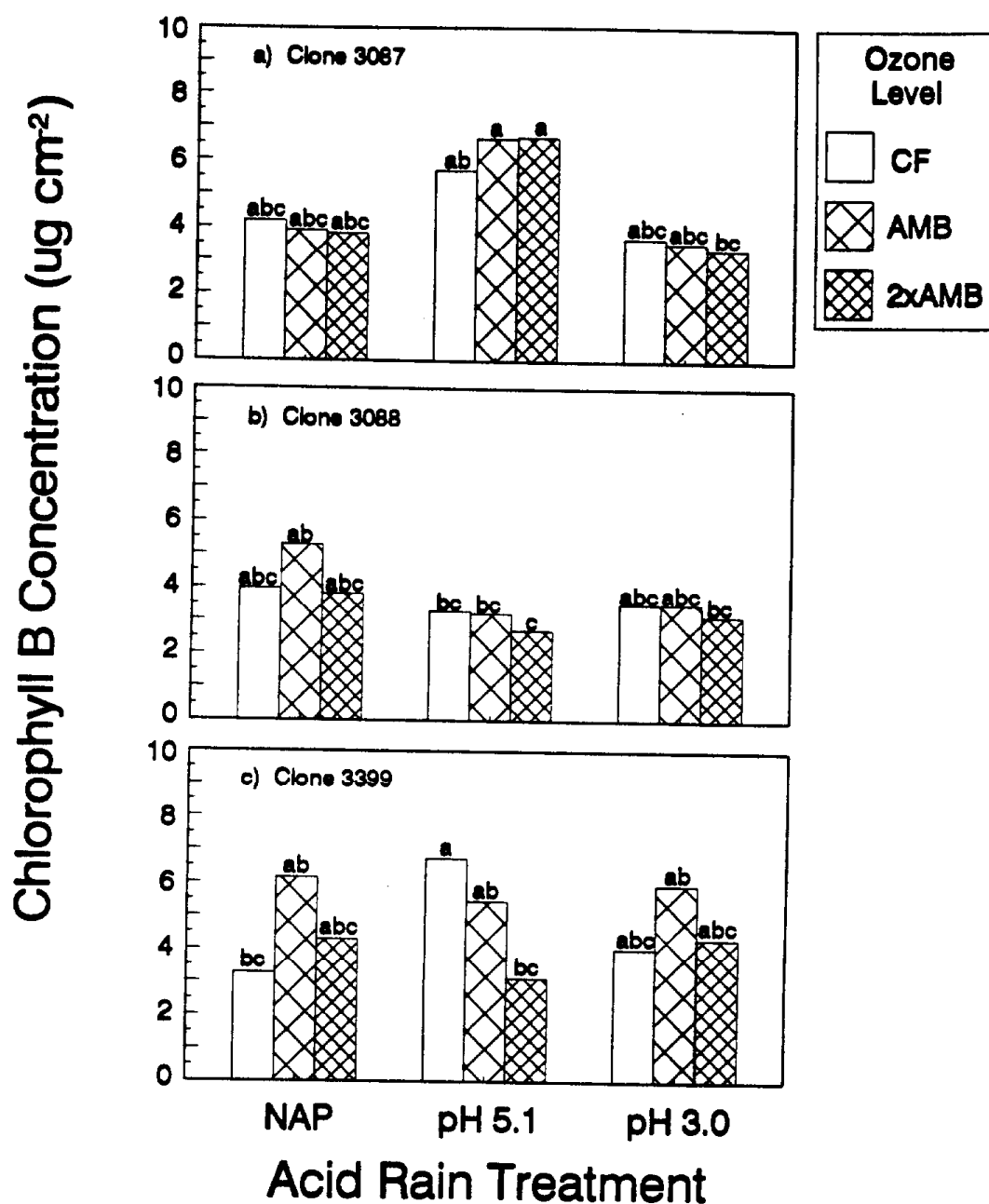


Figure 30. Late-summer foliar chlorophyll b concentration of *Pinus ponderosa* mature branches exposed to no acid rain (NAP), pH 5.1 rain or pH 3.0 rain and charcoal-filtered air (CF), ambient ozone (AMB) or twice ambient ozone (2xAMB) for genotypes a) 3087, b) 3088 and c) 3399. Among figures a-c, bars denoted by a common letter do not differ at the  $p=0.05$  level of significance.

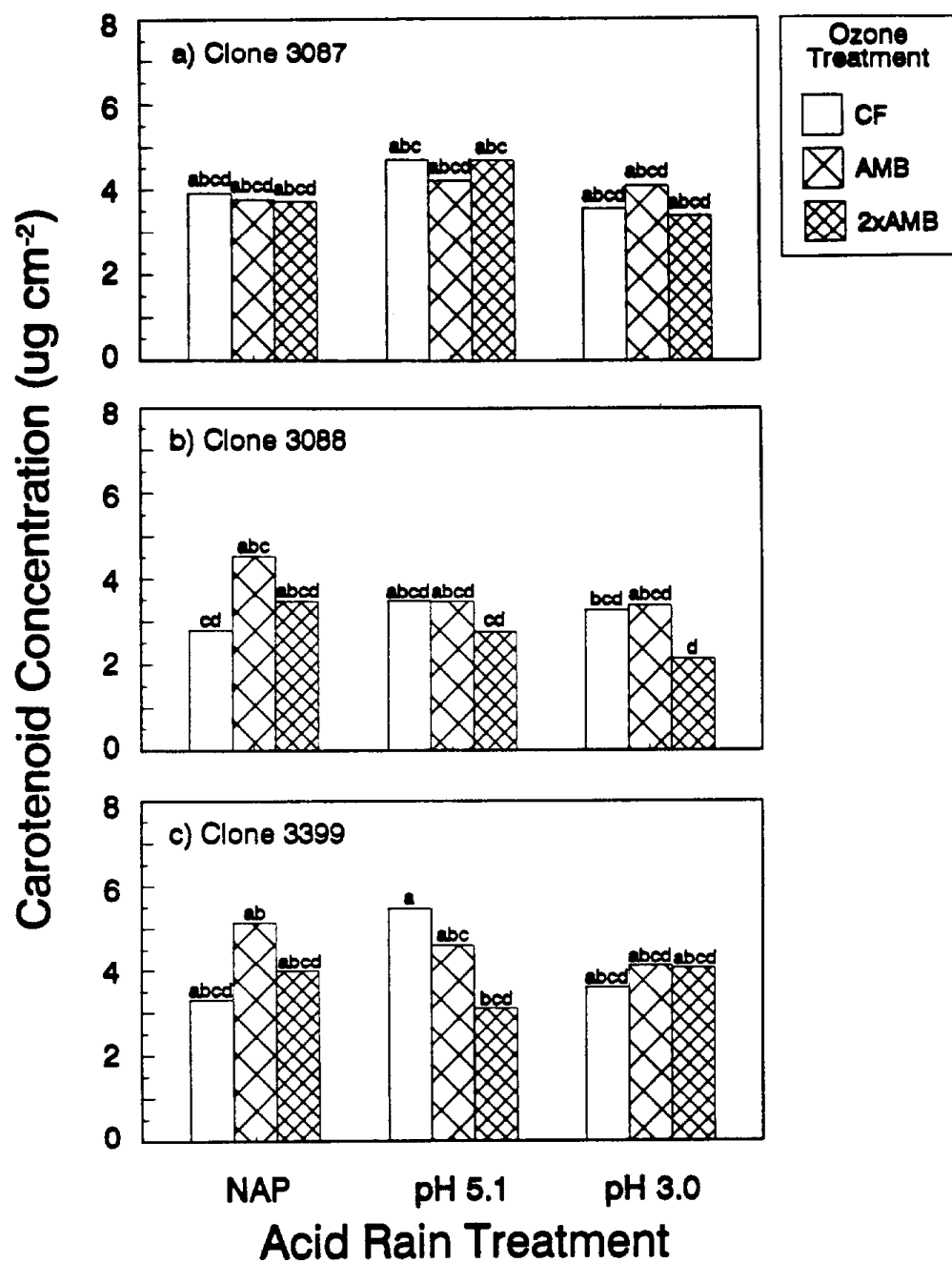


Figure 31. Late-summer foliar carotenoid concentration of *Pinus ponderosa* mature branches exposed to no acid rain (NAP), pH 5.1 rain or pH 3.0 rain for genotypes a) 3087, b) 3088 and c) 3399. Among figures a-c, bars denoted by a common letter do not differ at the  $p=0.05$  level of significance.

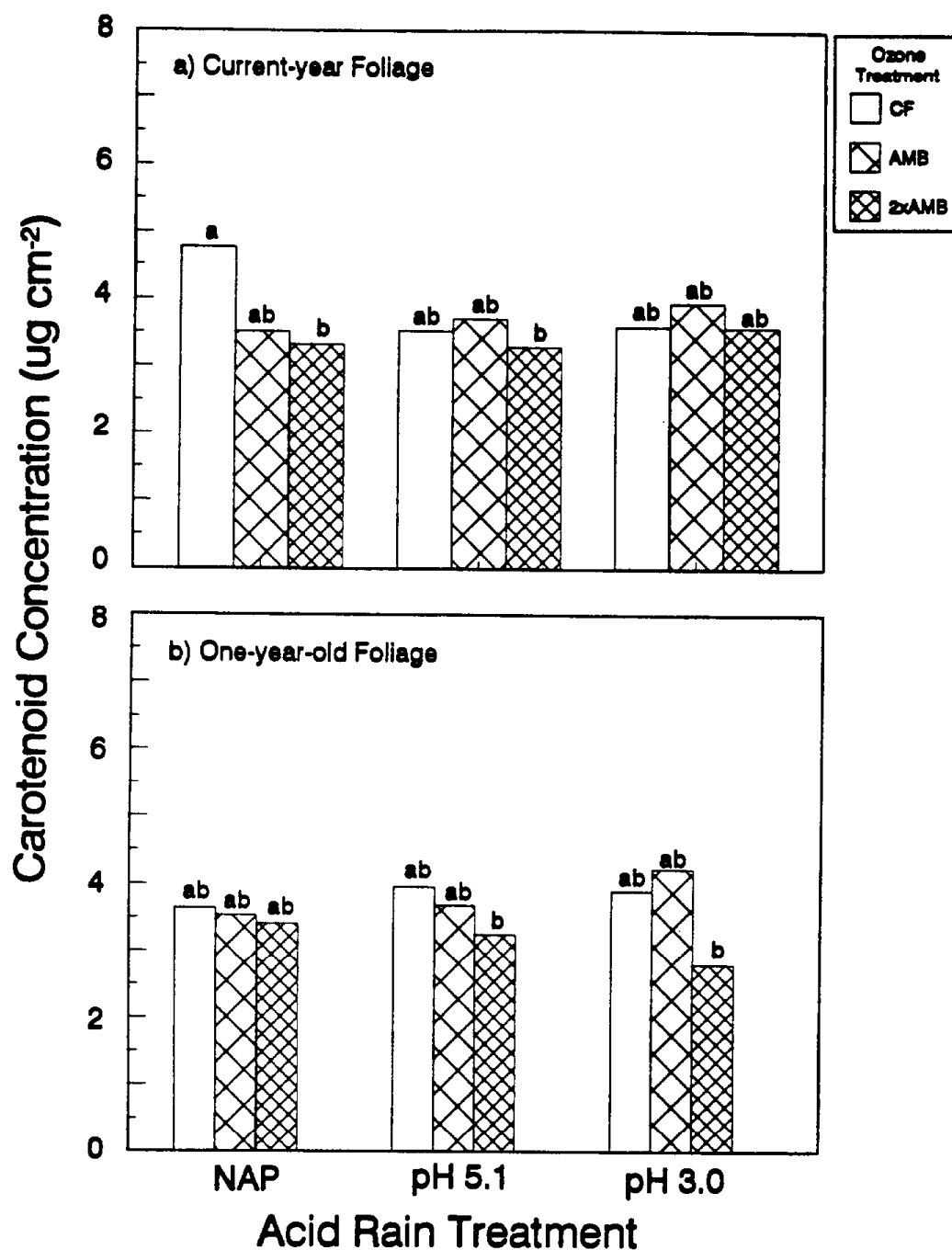


Figure 32. Late-summer carotenoid concentration of *Pinus ponderosa* seedling a) current-year foliage and b) one-year-old foliage exposed to charcoal-filtered air (CF), ambient ozone (AMB) or twice ambient ozone (2xAMB) and no acid rain (NAP), pH 5.1 rain (pH 5.1) or pH 3.0 rain (pH 3.0). Between figures a and b, bars denoted by a common letter do not differ at the  $p=0.05$  level of significance.

## F. Morphology and Growth

The growth of seedlings and mature branches was assessed by periodic measurement of seedling diameter and height or branch length and branch diameter. Initial seedling measurements were made on February 5, 1992 and August 21, 1991 for diameter and height, respectively. Initial branch length measurements were made on February 4, 1992 and initial branch diameter measurements were made August 20, 1991. Final measurements were made November 23 and November 24, 1992, for seedlings and branches, respectively. Final measurements coincided with termination of the ozone exposures.

### 1. Mature Branch Growth

#### a. Seasonal growth over all pollutants and genotypes (M)

##### i. Diameter

Diameter growth of mature branches occurred predominantly in two stages (Figure 33). The first stage, from August 1991 through January, 1992, accounted for 36 percent of the cumulative diameter growth and was characterized by an average rate of 0.21 percent  $d^{-1}$ . The second growth period occurred from late-April through mid-September with an average rate of 0.08 percent  $d^{-1}$ . Total percent diameter growth averaged 47.3 percent at the end of the study.

##### ii. Length

Much of the increase in branch length occurred from early-March through May, 1992 (Figure 33). During this period the average cumulative elongation was 30.5 percent and occurred at a rate of 0.36 percent  $d^{-1}$ . Following the initial, rapid phase of elongation, branch elongation slowed substantially (0.03-0.05 percent  $d^{-1}$ ) and tended to terminate by early November. Cumulative percent elongation averaged 37.2 percent for 1992.

#### b. Seasonal variation in genotype effect (M x G)

##### i. Diameter

There was significant variation in the seasonal pattern of diameter growth among the three genotypes ( $p=0.006$ , Table 41). As indicated in Figure 34a, early diameter growth by genotype 3399 tended to be less than that for either genotype 3087 or 3088. By the end of March, cumulative percent diameter growth for genotypes 3399, 3087 and 3088 was 29.8, 35.7 and 39.2 percent. Subsequent growth for genotypes 3087 and 3088 would total 9.3 and 11.4 percent, respectively. In contrast, continued diameter growth by genotype 3399 would average an additional 17.9 percent. As a result, by seasons end,

there was little difference in total percent diameter growth among mature branches of the three genotypes as mean values ranged from 45.1 to 49.7 percent.

ii. Length

Early season branch elongation did not differ among genotypes (Figure 34b). Significant differences in elongation among genotypes were established between late-April and late-May. By June, cumulative branch growth by genotype 3399 was 5 to 6 percent greater than that for either genotype 3087 or 3088. This relative difference in cumulative branch elongation was maintained over the remainder of the study (Figure 34b). The measurement period  $\times$  genotype effect on branch elongation was very highly significant ( $p < 0.001$ , Table 41).

c. Ozone effect (O)

Mature branch elongation differed significantly among ozone treatments as indicated by a significant ozone effect ( $p = 0.026$ , Table 41). When averaged over all measurement dates, acid rain treatments and genotypes, mean branch growth was 27.0, 29.0 and 19.2 percent for the CF, AMB and 2xAMB treatments, respectively. Thus, branch elongation was significantly less under the 2xAMB treatment relative to either the CF or AMB treatment.

d. Seasonal variation in ozone effect (M  $\times$  O)

i. Diameter

Throughout the study period there was a consistent trend for the greatest cumulative percent diameter to occur for branches receiving AMB ozone and for the lowest values to be associated with the 2xAMB ozone (Figure 35a). Through May, ozone treatment level means did not differ by more than 4.1 percent and values for the CF and 2xAMB treatments were very similar. Subsequently, differences between diameter growth in the AMB and 2xAMB treatments increased and the values for the CF treatment were either closer to the values for AMB or distinctly intermediate to the values for the AMB and 2xAMB treatments. Mean differences for the AMB and 2xAMB treatments increased to 11.4 percent at the end of the study. In spite of increasing mean separation, differences among ozone treatments were not significant for any single measurement date. The significant measurement period  $\times$  ozone interaction ( $p < 0.001$ , Table 41) is the result of seasonal variation in the relative difference among treatment level means over the study period.

## ii. Length

Differences in cumulative branch elongation were not consistent throughout the study period as indicated by a significant measurement period x ozone effect ( $p < 0.001$ , Table 41). From February through March, there was no significant difference in branch elongation among ozone treatments (Figure 35b). Between the end of March and the third week of April, branch growth in the 2xAMB treatment was substantially less than that for the CF and AMB treatments resulting in a difference of 6 to 7 percent cumulative elongation by early May. From May to the end of the study, percent growth rates were 0.040, 0.053 and 0.028 for the CF, AMB and 2xAMB treatments, respectively. At the end of the study, cumulative percent elongation for the 2xAMB treatment was 10.7 and 14.8 percent less than that of the CF and AMB treatments, respectively.

## e. Seasonal variation in the interactive effect of acidic rain and ozone (M x A x O)

There was a significant measurement period x ozone x acid rain interaction effect on mature branch elongation ( $p = 0.004$ , Table 41). The seasonal pattern of branch elongation as a function of acid rain treatment is illustrated for the CF, AMB and 2xAMB ozone treatments, respectively, in Figures 36a-36c. In all cases, a similar seasonal pattern of growth is observed with maximal rates of branch extension occurring through March, followed by slower growth through the end of the study period. Generally, treatment differences emerged before the end of May and were maintained relatively constant over the remainder of the study period.

For branches exposed to charcoal-filtered air, there was less than 2 percent difference in mean branch elongation between the NAP and AMB treatments (Figure 36a). Relative to the other rain treatments, branches exposed to pH 3.0 rain elongated approximately 11 percent less over the study period (Figure 36a).

When exposed to AMB ozone, elongation was greatest for pH 5.1 rain, lowest for pH 3.0 rain and intermediate for NAP (Figure 36b). Regardless of relatively large differences in means for the pH 5.1 and pH 3.0 treatments (14 to 24 percent from April through November), there was no significant effect of acid rain treatment for branches exposed to AMB ozone.

Under 2xAMB exposure, branch elongation was greatest, and nearly equal, for the pH 3.0 and NAP treatments (Figure 36c). Elongation by branches exposed to pH 5.1 rain was 9 to 10 percent less by the end of the study.

In comparing branch elongation response for each acid rain treatment, three different rankings of ozone treatment means are evident (Figures 36a-c). For the NAP treatment, branch elongation for the CF and AMB treatments were nearly identical and 10 to 12 percent greater than that for 2xAMB. When exposed to pH 5.1 rain, mean elongation was greatest for the AMB treatment and least for the 2xAMB treatment (55 and 23 percent, respectively, at the end of the study). Under the most acidic rain, there was less than 2 percent difference in total branch elongation among the three ozone treatments.

## 2. Seedling Growth

### a. Seasonal growth over all pollutants and genotypes (M)

#### i. Diameter

From August 1991 to November 1992, average seedling diameter increased by 104 percent (Figure 37). Although diameter growth occurred continuously during the study, the greatest rates of growth occurred from August to September, 1991 (0.40 percent  $d^{-1}$ ); from February to March 1992 (0.51 percent  $d^{-1}$ ); and from June through mid-September, 1992 (0.40 percent  $d^{-1}$ ) (Figure 37). These periods of rapid diameter growth occurred prior to shoot expansion in the spring and following termination of rapid shoot elongation in the early-summer.

#### ii. Stem Height

Seedling height growth began in early March 1992 and continued throughout the study period (Figure 37). Most rapid seedling height growth occurred from early March through early May at an average rate of 0.77 percent  $d^{-1}$ . From mid-May through mid-October, seedling height growth averaged 0.12 percent  $d^{-1}$ .

### b. Seasonal variation in genotype effect (M x G)

#### i. Diameter

There was a significant measurement period x genotype interaction effect on seedling diameter growth ( $p=0.036$ , Table 42). Throughout most of the study period there was little difference in cumulative percent diameter increase among the three half-sib genotypes (Figure 38a). But, at the final measurement in November, 1992, percent diameter growth for genotype 3088 (112 percent) was greater than that for genotype 3087 (97 percent). The final percent growth for genotype 3399 (103 percent) was intermediate and did not differ significantly from that for the other genotypes.

#### ii. Stem Height

From late-April through mid-November, 1992, cumulative height growth by half-sib seedlings of genotypes 3088 and 3399 was significantly greater than that for genotype 3087 (Figure 38b). By the end of the study, percent height growth for the genotypes 3088, 3399 and 3087 was 79.6, 75.6 and 64.2 percent, respectively. Prior to mid-April, there was no significant difference in cumulative percent height growth among the three genotypes. The onset of significant genotype differences was evident as a very highly significant ( $p<0.001$ ) measurement period x genotype term in the RMANOVA (Table 42).

c. Acid rain effect and seasonal variation in acid rain effect (A, M x A)

Seedling diameter growth was significantly affected ( $p=0.007$ ) by acid rain treatment (Table 42). Cumulative percent growth, averaged over all measurement periods was 25.1, 25.8 and 34.2 percent for the NAP, pH 5.1 and pH 3.0 treatments, respectively. Diameter growth for seedlings exposed to pH 3.0 rain was greater than that for seedlings exposed to the other two treatments.

Although acid rain was a significant effect over the study period, the significance of treatment effects varied with time as indicated by the significant measurement period x acid rain term ( $p=0.036$ , Table 42). While, treatment mean values for the pH 3.0 treatment were consistently greater than those for the pH 5.1 and NAP treatments, the greatest difference among treatment mean values occurred late in the study (Figure 39). At the final measurement, percent diameter growth was 117.8, 97.8 and 97.0 percent for the pH 3.0, pH 5.1 and NAP treatments, respectively.

Some caution must be used in assessing the magnitude of diameter growth enhancement by the pH 3.0 treatment. As illustrated in Figure 39, the average percent cumulative growth for the pH 3.0 treatment exceeded the values for the pH 5.1 and NAP treatments by 5 to 6 percent as early as November, 1991, prior to the application of acid rain exposures in 1992. If it is assumed that there was an inherent 5 to 6 percent difference in growth potential among acid rain treatments, then the magnitude of pH 3.1 exposure effect on diameter is probably 14 to 15 percent rather than the 20 percent implied by measurements made at the end of the study.

There were no significant effects of acid rain or measurement period x acid rain evident for seedling height growth (Table 42).

d. Seasonal variation in the interactive effect of ozone and genotype (M x O x G)

There was a significant ( $p=0.014$ ) interaction effect of measurement period, ozone and genotype on seedling height growth (Table 42). Figures 40a-40c illustrate the seasonal variation in seedling height growth response to ozone for genotypes 3087, 3088 and 3399, respectively. These data clearly suggest a differential sensitivity to ozone among seedlings of three genotypes.

For genotype 3087, there was little seasonal variation in the ranking of mean values among ozone treatments. The lowest cumulative growth, throughout the study period, was observed for the AMB treatment (Figure 40a). By late 1992, growth under the CF treatment tended to be the greatest, yet differences among treatment means were not statistically significant.

Height growth by seedlings of genotype 3088 was substantially reduced for the 2xAMB treatment, relative to the CF and AMB treatments, from late-May, 1992 through to the end of the study (Figure 40b). Following initial shoot elongation in March and April, height growth averaged 0.130 and 0.144 percent  $d^{-1}$  for the CF and AMB treatments while the 2xAMB rate of growth was only 0.088 percent  $d^{-1}$ . By the end of the study period, cumulative percent height growth was 85.9, 83.9 and 69.1 percent for the AMB, CF and 2xAMB treatments, respectively.

Seedling height growth for genotype 3399 varied little among ozone treatments (Figure 40c). Although there was a slight tendency for seedlings exposed to the AMB treatment to grow the least, differences in cumulative percent height growth among the ozone treatments were less than 6 percent for any measurement period.

Table 41. Summary of mature branch diameter and length growth repeated measures ANOVA.

Mature Branch Growth RMANOVA								
Source	Basal Diameter				Length			
	DF	MS	F	Pr>F	DF	MS	F	Pr>F
Between Subj.								
Genotype (G)	2	0.1511	0.78	0.485	2	0.3707	1.94	0.200
Acid Rain (A)	2	0.4339	2.25	0.161	2	0.2250	1.18	0.352
G x A	4	0.6320	3.28	0.064	4	0.0619	0.32	0.855
Error I	9	0.1926			9	0.1934		
Within Subj.								
Ozone (O)	2	0.1684	0.63	0.548	2	0.7162	4.50	0.026
G x O	4	0.2753	1.02	0.424	4	0.2009	1.26	0.321
A x O	4	0.1685	0.62	0.652	4	0.3355	2.11	0.122
G x A x O	8	0.2352	0.87	0.558	8	0.0357	0.22	0.982
Error II	18	0.2703			18	0.1591		
Month (M)	9	0.1595	48.11	<0.001	11	0.7322	227.9	<0.001
M x G	18	0.0077	2.31	0.006	22	0.0151	4.68	<0.001
M x A	18	0.0047	1.41	0.148	22	0.0033	1.02	0.448
M x G x A	36	0.0013	0.38	0.999	44	0.0016	0.51	0.994
Error III	81	0.0033			99	0.0032		
M x O	18	0.0063	2.86	<0.001	22	0.0119	4.07	<0.001
M x G x O	36	0.0032	1.46	0.059	44	0.0032	1.09	0.334
M x A x O	36	0.0021	0.93	0.585	44	0.0052	1.78	0.004
M x G x A x O	72	0.0013	0.59	0.994	88	0.0012	0.41	1.000
Error IV	162	0.0022			198	0.0029		

Table 42. Summary of seedling diameter and height growth repeated measures ANOVA.

Seedling Growth RMANOVA								
Source	Basal Diameter				Height			
	DF	MS	F	Pr>F	DF	MS	F	Pr>F
Between Subj.								
Acid Rain (A)	2	1.4015	6.01	0.007	2	0.2236	0.53	0.595
Ozone (O)	2	0.2608	1.12	0.342	2	0.1186	0.28	0.757
A x O	4	0.2216	0.95	0.451	4	0.2149	0.51	0.729
Error I	27	0.2333			27	0.4220		
Within Subj.								
Genotype (G)	2	0.1006	0.79	0.458	2	1.8326	12.10	<0.001
A x G	4	0.0881	0.69	0.599	4	0.3911	2.58	0.047
O x G	4	0.0367	0.29	0.884	4	0.1872	1.24	0.307
A x O x G	8	0.0782	0.62	0.760	8	0.0924	0.61	0.765
Error II	54	0.1269			54	0.1514		
Month (M)	11	9.1863	633.97	<0.001	11	5.4951	612.6	<0.001
M x A	22	0.0243	1.68	0.030	22	0.0047	0.52	0.719
M x O	22	0.0180	1.24	0.212	22	0.0079	0.88	0.483
M x A x O	44	0.0135	0.93	0.598	44	0.0061	0.68	0.710
Error III	297	0.0145			297	0.0090		
M x G	22	0.0172	1.62	0.036	22	0.0220	4.36	<0.001
M x A x G	44	0.0083	0.78	0.847	44	0.0057	1.13	0.262
M x O x G	44	0.0069	0.65	0.959	44	0.0079	1.56	0.014
M x A x O x G	88	0.0082	0.77	0.935	88	0.0032	0.65	0.994
Error IV	594	0.0106			594	0.0050		

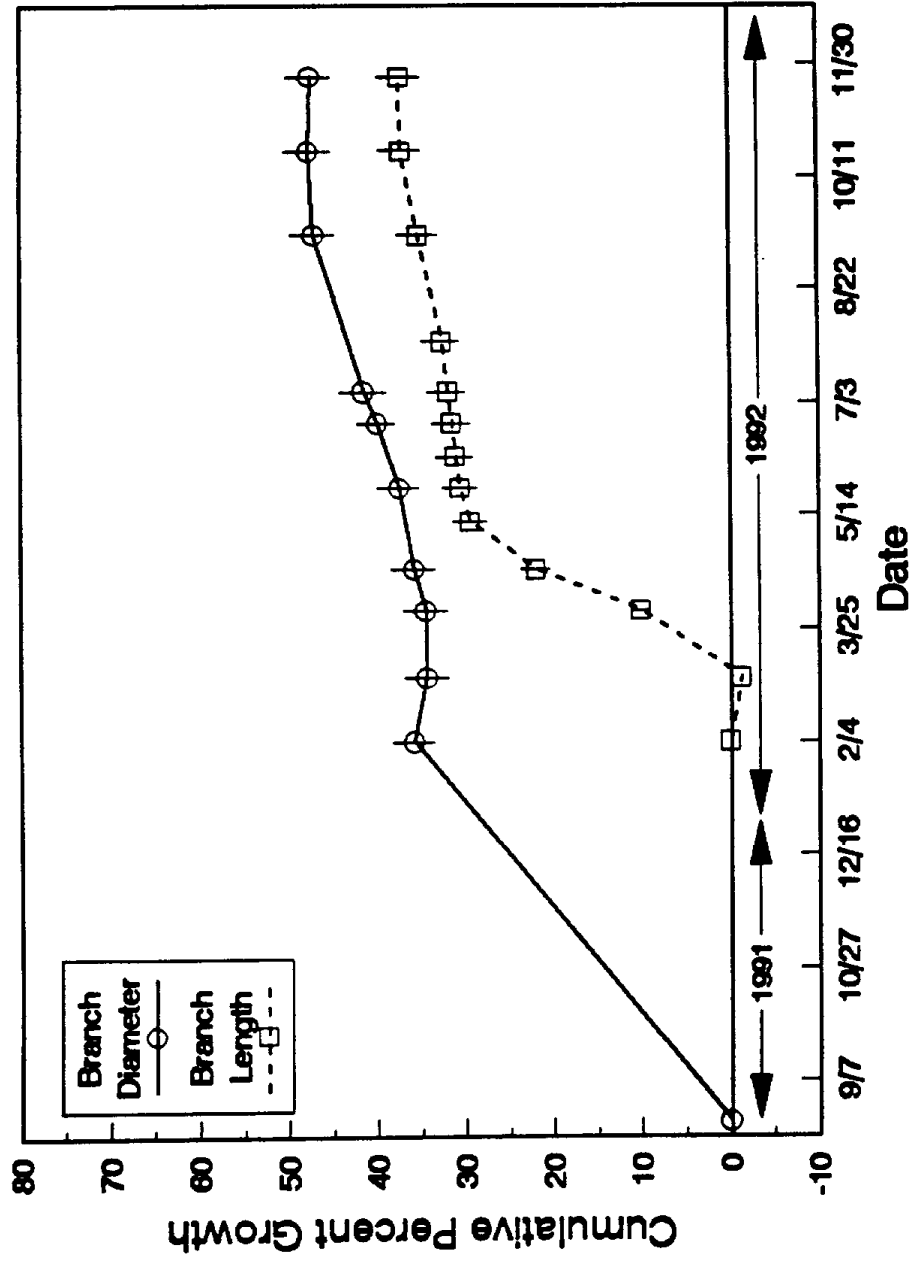


Figure 33. Diameter and length growth of *Pinus ponderosa* mature branches. Values represent mean cumulative percent growth relative to initial diameter or length of previous years branch segment. Vertical lines represent one standard error of the mean.

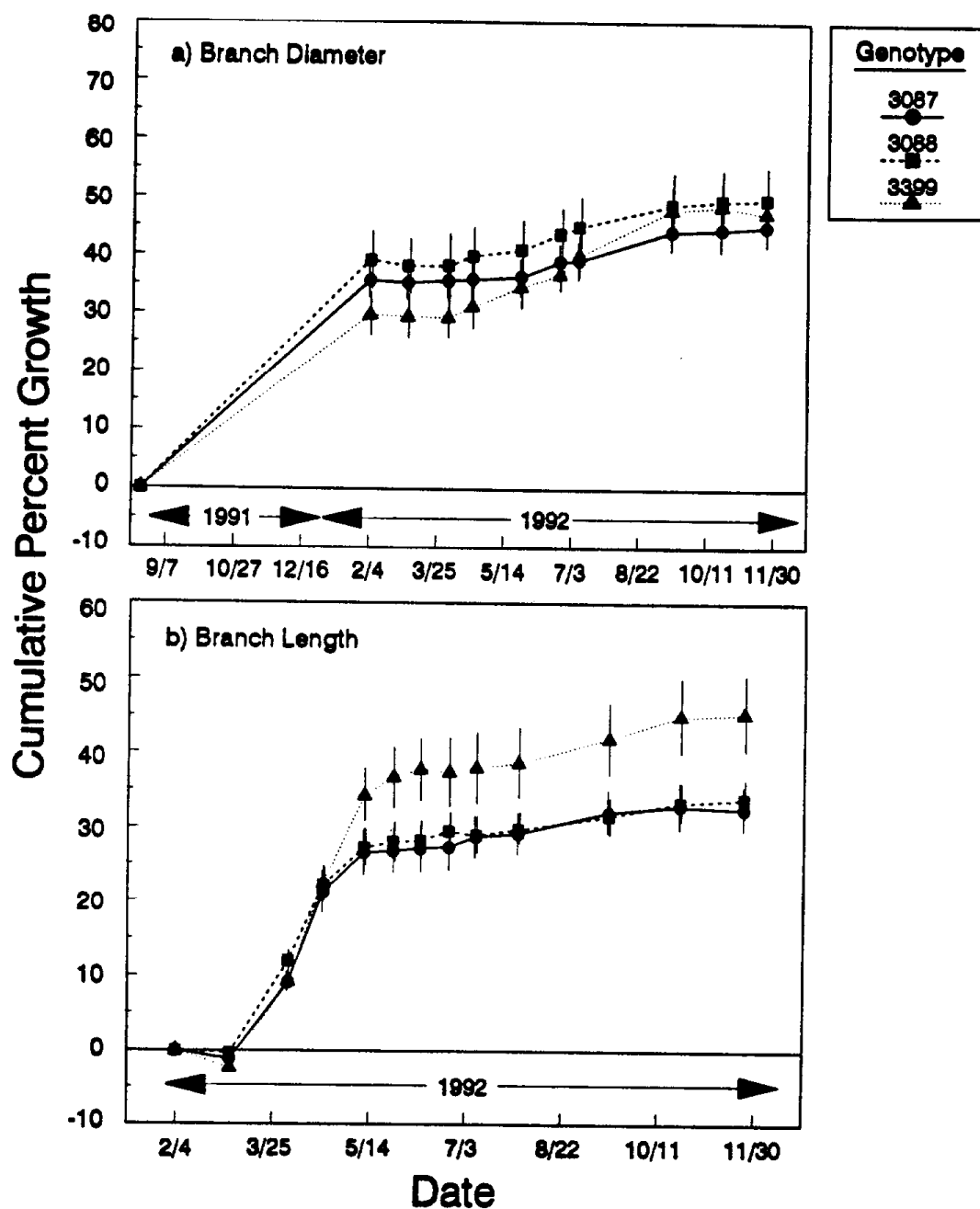


Figure 34. Mature branch a) diameter and b) length growth for *Pinus ponderosa* of genotypes 3087, 3088 and 3399. Values represent mean cumulative percent growth relative to initial diameter or length of previous years branch segment. Vertical lines represent one standard error of the mean.

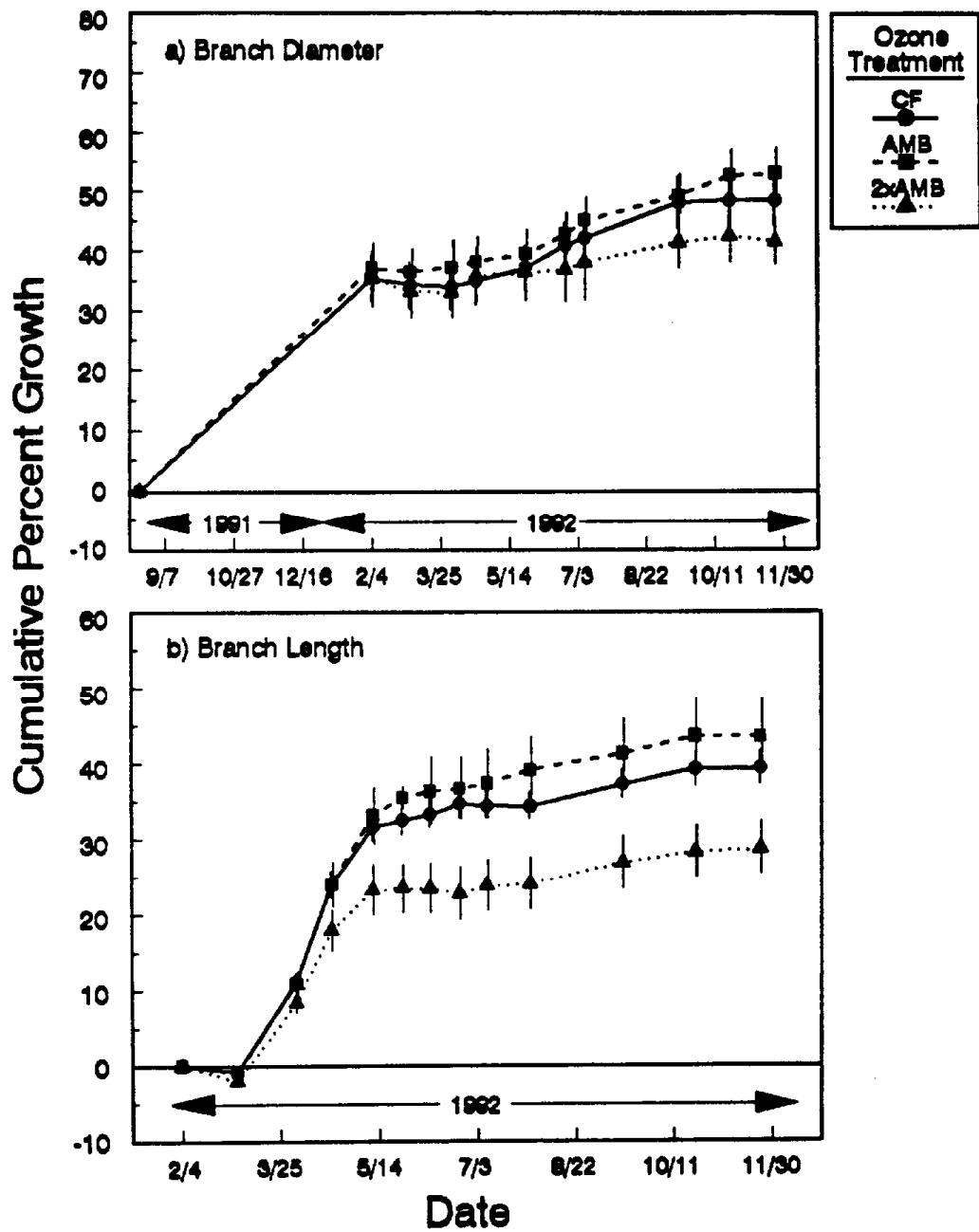


Figure 35. Mature branch a) diameter and b) length growth for *Pinus ponderosa* exposed to charcoal-filtered air (CF), ambient ozone (AMB) or twice ambient ozone (2xAMB). Values represent mean cumulative percent growth relative to initial diameter or length of previous years branch segment. Vertical lines represent one standard error of the mean.

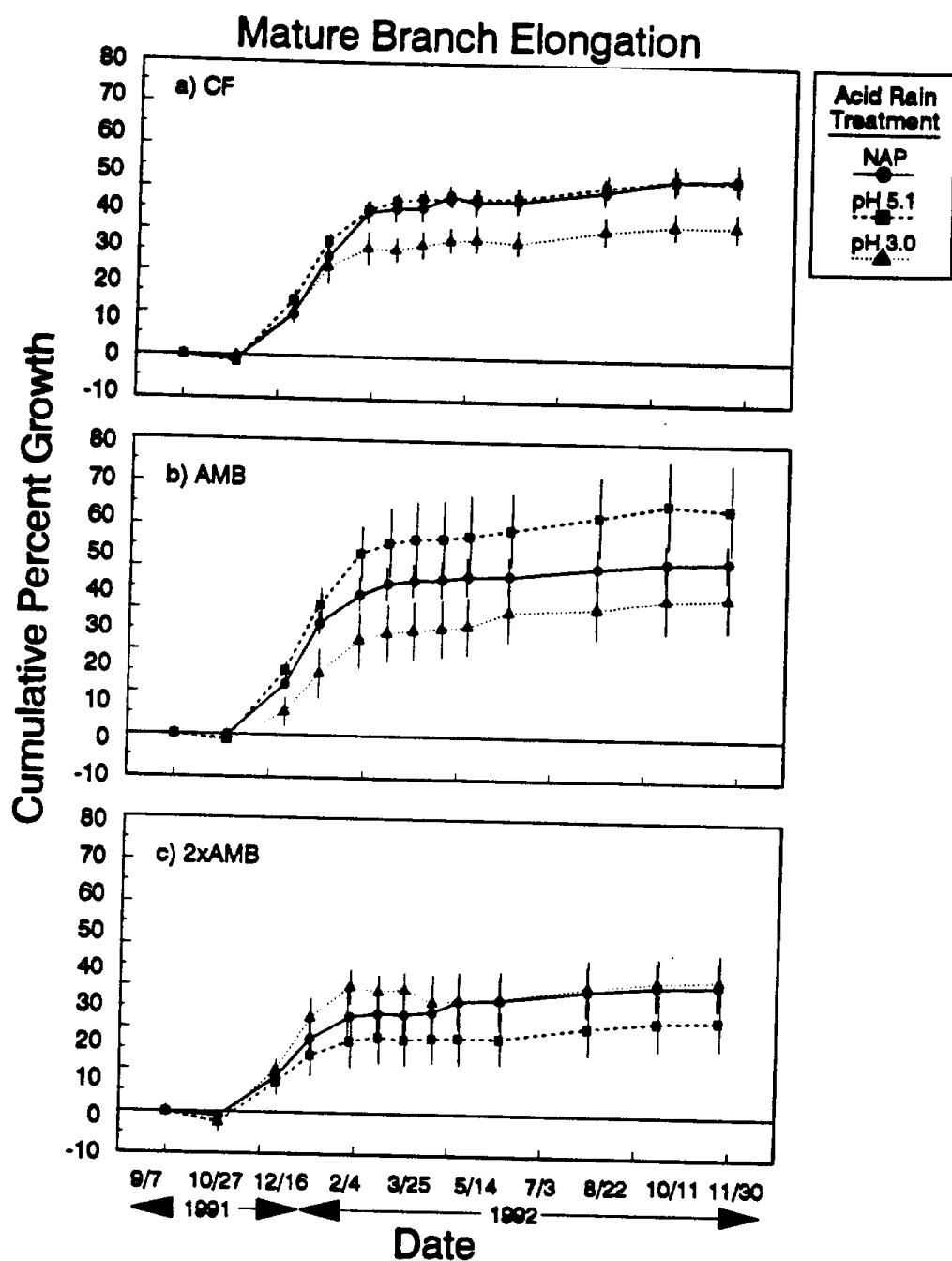


Figure 36. Mature branch elongation for *Pinus ponderosa* exposed to no-acid rain (NAP), pH 5.1 rain (pH 5.1), or pH 3.0 rain (pH 3.0) and a) charcoal-filtered air (CF), b) ambient ozone (AMB), or c) twice ambient ozone (2xAMB). Values represent mean cumulative percent growth relative to the length of the previous years branch segment. Vertical lines represent one standard error of the mean.

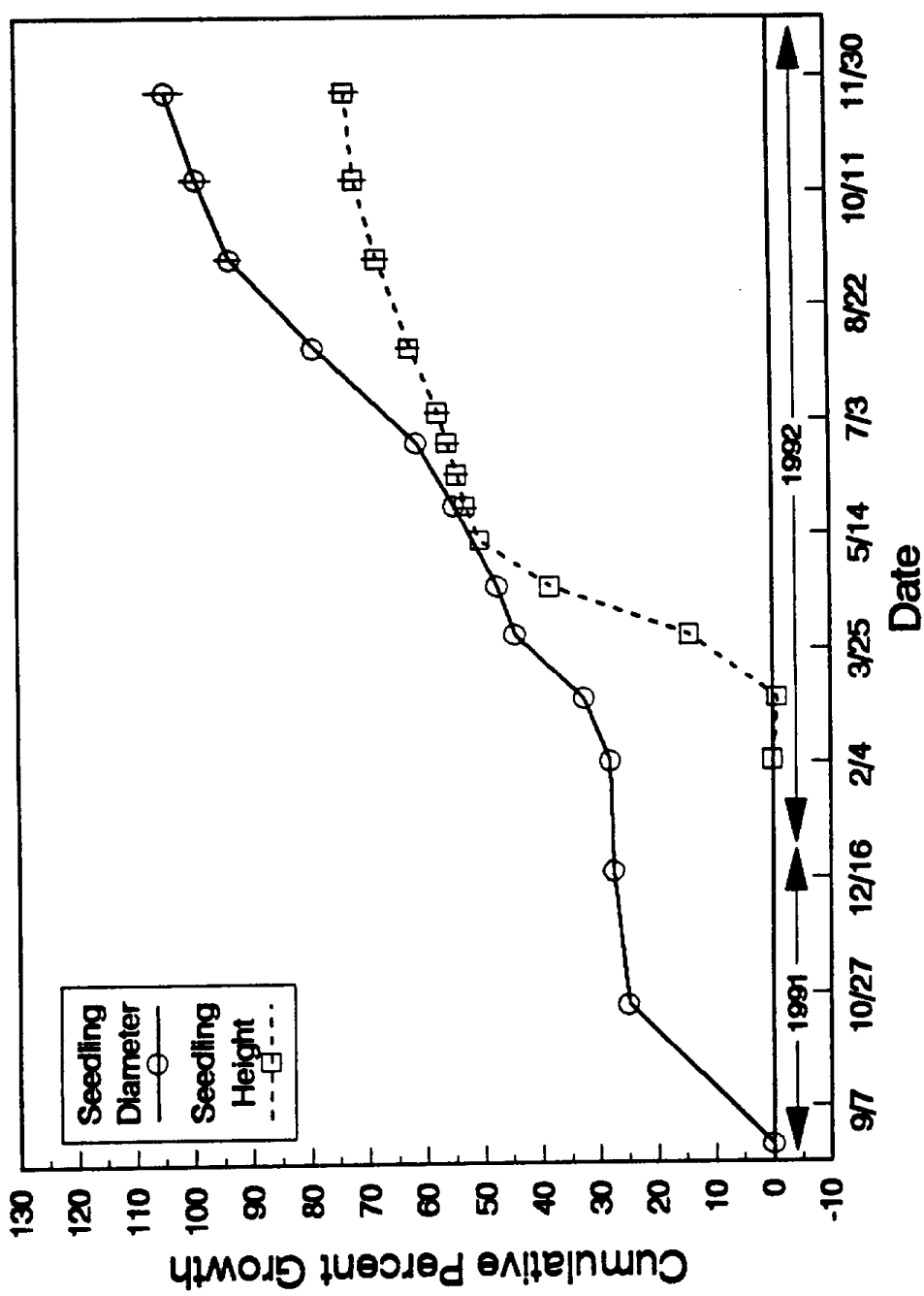


Figure 37. Diameter and height growth of *Pinus ponderosa* seedlings. Values represent mean cumulative percent growth relative to initial diameter or the total height prior to elongation of the current-year shoot. Vertical lines represent one standard error of the mean.

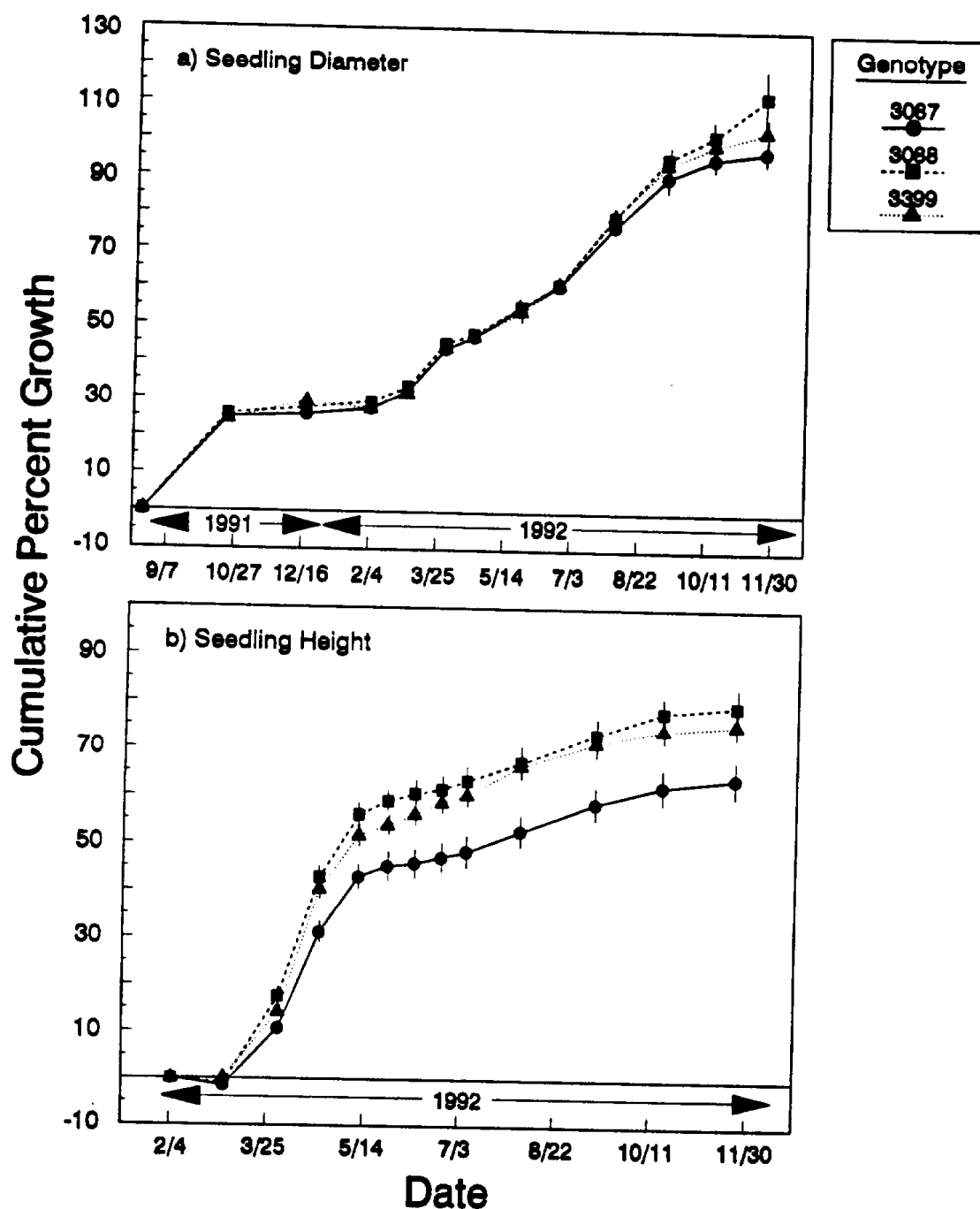


Figure 38. Seedling a) diameter and b) height growth for *Pinus ponderosa* half-sib genotypes 3087, 3088 and 3399. Values represent mean cumulative percent growth relative to initial diameter or the total height prior to elongation of the current-year shoot. Vertical lines represent one standard error of the mean.

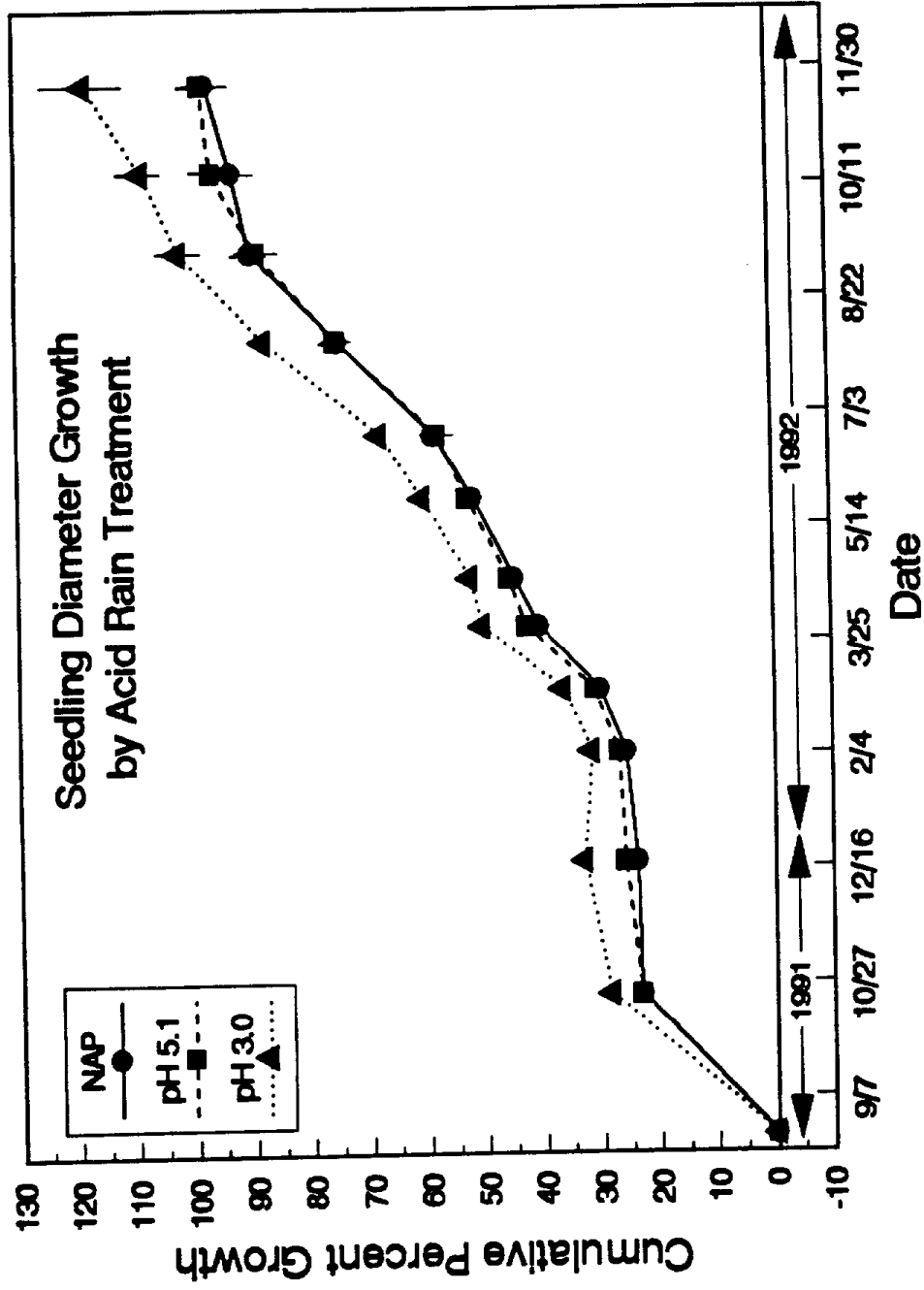


Figure 39. Seedling diameter growth for *Pinus ponderosa* exposed to no acid rain (NAP), pH 5.1 rain (pH 5.1) or pH 3.0 (pH 3.0). Values represent mean cumulative percent growth relative to initial stem diameter. Vertical lines represent one standard error of the mean.

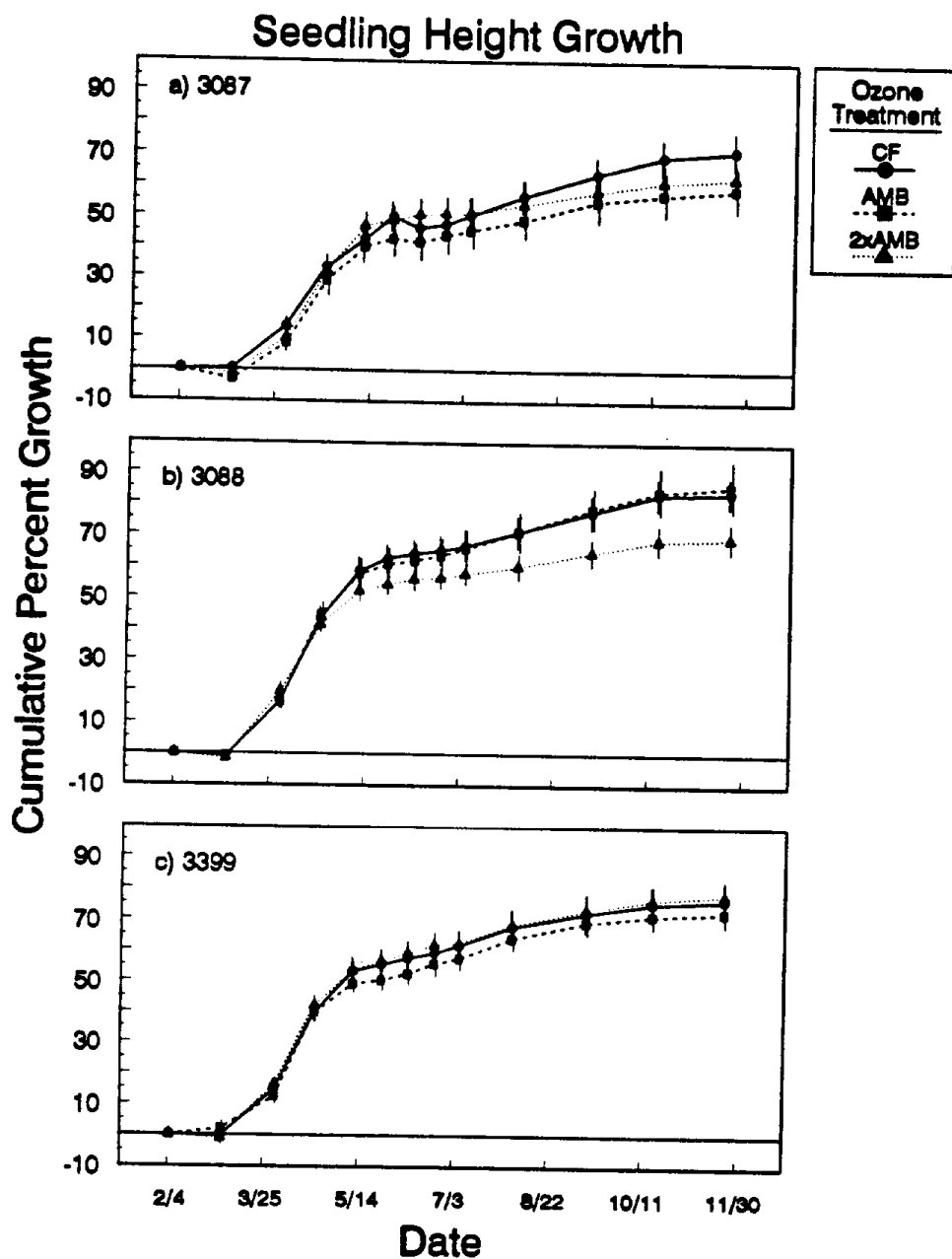


Figure 40. Seedling height growth for *Pinus ponderosa* of half-sib genotypes a) 3087, b) 3088, and c) 3399 when exposed to charcoal-filtered air (CF), ambient ozone (AMB) or twice ambient ozone (2xAMB). Values represent mean cumulative percent growth relative to the total height prior to elongation of the current-year shoot. Vertical lines represent one standard error of the mean.

#### IV. Discussion

##### A. Overall response to long-term acidic rain and ozone exposure

In our study, there was a lack of evidence for substantial acid rain impact on stomatal conductance by one-year-old foliage of either mature tree branches or seedlings. However, observed mid-day  $g_s$  values for current-year mature branch foliage were consistently lower (from 2 to 18 percent) for tissue exposed to pH 3.0 rain than for tissue exposed to either no acid rain or pH 5.1 acid rain. This reduction in  $g_s$  may have resulted from an alteration in tissue water relations. Exposure to acid rain has been shown to cause degradation of the cuticular waxes found on pine needles (Halopainen and Nygren 1989). It has been suggested that the degenerated wax structures may occlude stomates and thereby reduce  $g_s$  (Sauter and Voss 1986). Using SEM, we observed substantial epicuticular wax degradation and possible stomatal occlusion on one-year-old ponderosa pine mature branch foliage exposed to acid rain and ozone during a 1990 study at the CAPACC site (Newman, unpublished data). No change in cuticular resistance to water flux was observed for red spruce needles treated with acid mist (Eamus *et al.* 1989).

A second possible mechanism for decreased  $g_s$  with acidic rain exposure would be a decrease in the amount of guard cell osmoticum resulting from nutrient element leaching, particularly  $K^+$ . It has been hypothesized that exposure to acid precipitation may lead to increased leaching of  $K^+$ ,  $Mg^{2+}$  and  $Ca^{2+}$  from foliage (Lovett *et al.* 1985). There is inconsistent evidence regarding acid precipitation induced  $K^+$  leaching from pine foliage as substantial reductions have been observed for Scots pine (*Pinus sylvestris*) (Skeffington and Roberts 1985) while (MacDonald *et al.* 1986) observed no leaching for seedlings of jack pine (*Pinus banksiana*) and Lee *et al.* (1990) observed an increase in foliar  $K^+$  concentration in red spruce seedlings.

Carbon metabolism plays an important role in stomatal function as it is responsible for the availability of starch from which is derived many of the organic acids that serve to balance the ionic charge as  $K^+$  flux occurs to and from the guard cells (Jones 1983). Should acid rain exposure cause a reduction in carbon assimilation or an alteration of carbon partitioning, stomatal function may be disrupted. Holopainen and Nygren (1989) found no substantial changes in photosynthetic performance of foliage shown to have slight alterations in chloroplast structure induced by  $K^+$  deficiencies. Eamus *et al.* (1989) observed alterations in the tissue water status of red spruce (*Picea abies*) seedlings exposed to acid rain consisting of reduced relative water content and maximum turgor that coincided with reduced carbon fixation. Although, in this study,  $P_n$  rates for current-year mature branch foliage exposed to pH 3.0 rain tended to be 6 to 11 percent lower than for the NAP and pH 5.1 treatments, there was not a strong indication that the decrease in net carbon uptake of was associated with the observed decrease in  $g_s$  at pH 3.0.

A consistent trend of decreasing  $P_n$  with increasing ozone exposure was observed for both seedlings and mature branches and for both current-year and one year-old foliage. Significant decreases in  $P_n$  with elevated ozone were present only for one-year-

old mature branch foliage. The 10 to 14 percent decrease for seedlings and the 19 percent decrease observed for mature branches is similar to the 10 percent reduction in photosynthetic capacity reported by Coyne and Bingham (1981, 1982) for ozone injured ponderosa pine growing in the San Bernardino National Forest. Sasek and Richardson (1989) reported reductions in photosynthetic capacity of 21 to 27 percent for loblolly pine exposed to twice ambient ozone (29 ppb 12-h mean). Both groups concluded that the reduction in photosynthetic capacity was due to changes in light harvesting and biochemical activities and not due to alterations of stomatal limitation. In the present study, we observed trends for decreased  $g_s$  with increasing ozone exposure for both life-stages and both foliage age-classes. Although significant ozone effects for  $g_s$  were not present, the magnitude of the  $g_s$  declines (3 to 14 percent for mature branches and from 6 to 9 percent for seedlings) was similar to the relative decrease in  $P_n$ .

In contrast, Beyers *et al.* (1992) observed increased photosynthetic capacity for current-year ponderosa pine foliage exposed to 1.5 times ambient ozone under well-watered conditions. They attributed this relative increase to improved foliar nitrogen status arising from re-translocation of nitrogen from senescent foliage.

In late summer, significant decreases in ChlA, ChlB and Car were observed for seedlings and mature branches exposed to 2xAMB ozone relative to the CF and AMB treatments. Decreased foliar pigment concentration in response to ozone exposure have been observed for loblolly pine (Sasek *et al.* 1991, Sasek and Richardson 1989) and, as evident by chlorotic mottle, in ponderosa pine (Temple *et al.* 1992). Edwards *et al.* (1990) observed an increase in pigment concentration for loblolly pine seedlings exposed to ozone although they suggest that rather than being a response to ozone, the increase may have occurred as the result of N fertilization as a by product of their methanogenic ozone generation. The observed decrease in chlorophyll pigment concentration may be an indication of altered chloroplast function and decreased light harvesting capacity for foliage exposed to elevated ozone.

Pines typically express changes in growth in the year following exposures to a stress. In this experiment, exposures began in late fall 1991. These early exposures could have had an impact on carbon partitioning and storage, and resulted in a modest impact in branch elongation for the 1992 growing season. Other studies have shown that exposing plants to episodic ozone events has been demonstrated to alter carbohydrate metabolism. For example, *Pinus strobus* has been shown to exhibit altered carbohydrate partitioning after experiencing ozone exposure levels of 10 and 20 ppm for periods of 7 and 21 days (Wilkinson and Barnes 1973). In these experiments a reduction was observed in the foliar content of the soluble sugar fraction (primarily sucrose), and an increase in the level of sugar-phosphate compounds which became exaggerated with prolonged exposure. Similar results were obtained in a companion study with *Pinus taeda*, an ozone tolerant species suggesting that alterations in carbohydrate metabolism is a uniform response to ozone exposure. In our study, branches of *P. ponderosa* experienced ozone levels similar to those used in the *P. strobus* and *P. taeda* study. Thus, the decreased branch elongation we observed could have been the result of reductions in carbon assimilation, or an alteration in carbon allocation which favored carbon sinks other than branch length (eg. foliar repair). The ozone exposures conducted in the late

fall of 1991 may have depleted carbon reserves that would have been available for growth in 1992. Such a metabolic alteration would modify carbon allocation between the source/sink and impact growth potential.

In contrast to the findings of Temple *et al.* (1993), we did not observe significant ozone effects on the diameter growth of ponderosa pine seedlings. Following 3 years cumulative ozone exposure they determined that exposure to elevated ozone resulted in increased growth of current-year foliage and stems at the expense of radial growth. Our data indicate that there was virtually no difference in current-year height growth and a non-significant decrease in seasonal diameter growth of approximately five percent. This difference among results is not surprising given that our study was conducted for a little more than one season. Substantial alterations of carbon allocation may be manifest to a greater degree with ozone exposure over multiple seasons.

#### B. Seasonality of response to acidic rain and ozone exposure

Regardless of acid rain exposure, ozone treatment or genotype, observed mid-day  $g_s$  values were greatest in the early season months of March and April and thereafter, exhibited various rates of decline through August. Much of the observed seasonality of  $g_s$  was due to increasing severity of microclimate including increasing mean temperatures and vapor pressure deficits. Superimposed upon the ambient environmental stress was variation associated with different genotypes or pollutant exposure. Early season differences in mid-day  $g_s$  between non-treated and pollutant exposed foliage varied among life-stages as one-year-old branch tissue exhibited a more rapid seasonal  $g_s$  decline for tissues exposed to 2xAMB ozone than for tissues subjected to the CF treatment. This trend was not observed in seedlings. Conversely, seedlings exposed to simulated rain of pH 3.0 exhibited a tendency for a more rapid seasonal decline in  $g_s$  relative to seedlings receiving no rain, while mature branches did not.

The observed reduction in  $g_s$  rates for mature branches exposed to 2xAMB ozone were interesting in that the large difference in  $g_s$  with respect to the AMB and CF treatments (25 to 35 percent less) was that it arose not because of a continued decline in  $g_s$  for the 2xAMB treatment, but because of a late-season increase in  $g_s$  for the CF and AMB treatments. This lack of  $g_s$  recovery with reduced environmental stress in the fall indicates that there was an alteration of stomatal response mechanisms under the high ozone treatment. It is unlikely that reductions in carbon assimilation accounted for this lack of  $g_s$  recovery as there was an observed increase in  $P_n$  for branches exposed to 2xAMB ozone from August to October that was proportional to the observed increases in  $P_n$  for branches receiving CF and AMB ozone treatments for the same period. Coyne and Bingham (1982) attributed seasonal reductions in  $g_s$  for ozone damaged one-year-old and two-year-old ponderosa pine foliage to accelerated senescence but they could not attribute their observations strictly to ozone damage as they lacked non-impacted controls. In our study, the increased  $g_s$  performance during the late-season does provide a strong basis for concluding that accelerated senescence due to ozone injury does occur for mature trees. Premature senescence in response to prolonged ozone exposure has also been demonstrated for ponderosa pine seedlings (Temple *et al.* 1992) and loblolly

pine seedlings (Stow *et al.* 1992). In ponderosa pine, early senescence was related to a reduction in leaf carbohydrate concentration (Miller *et al.* 1969). It should be noted that ozone induced senescence may differ from natural foliage ageing in that normal processes of starch and nutrient re-translocation may not always occur (Gunthardt-Goerg *et al.* 1993).

Distinct reductions in mid-day  $P_n$  existed for one-year-old foliage of mature branches at the time of first measurement in February, 1992. This is interesting in that it indicates that following two months exposure to ambient ozone, ozone exposure effects induced during fumigation at 2xAMB levels from late-August through November, 1991 persisted. Similar tendencies were also evident for one-year-old seedling foliage but the extent of  $P_n$  reduction for 2xAMB ozone, relative to AMB ozone, was not as great. The persistence of the ozone injury effect suggests that in the environment at the CAPACC site, substantial injury repair may not occur during the 2-3 month period of low ozone concentration occur during the winter. The fact that injury repair is not a speedy process is evident by the lack of an increase in  $P_n$  values for foliage exposed to 2xAMB ozone, relative to  $P_n$  for tissues exposed to CF or AMB, in July and August following a 31 d interruption in ozone fumigation.

Evidence that ozone injury in older foliage may alter carbon, and possibly nutrient, allocation patterns is suggested by the presence of reduced  $P_n$  rates at the time emergence in current-year foliage on seedlings exposed to 2xAMB ozone. This effect termed "carryover" was observed by Sasek *et al.* (1991) for multiple flushes of loblolly pine seedlings exposed to various ozone concentrations over a two-year period and by white pine seedlings (*Pinus strobus*) (Mann *et al.* 1980). This carryover effect is distinguished by ozone impaired  $P_n$  rates that do not recover over the winter and a subsequent reduction in potential  $P_n$  of newly emerging foliage. Sasek *et al.* suggest that the probable cause for such phenomenon is a lack of stored carbohydrates available to newly emerging tissue as a result of the reduced photosynthetic capacity of older foliage. Accelerated senescence in which stored carbohydrates and nutrients are not re-translocated (Gunthardt-Goerg *et al.* 1993) may also contribute to this phenomenon.

It is not known what impact the mid-season interruption of ozone exposure had on physiological and growth performance. It is apparent from the data that the 4-week interruption did not result in complete recovery from cumulative ozone impact as the relative difference in gas-exchange rates between the AMB and 2xAMB treatments was essentially the same in July, when ozonation resumed, as in June, when ozonation was temporarily discontinued. Gas exchange rates for one-year-old foliage of both mature branches and seedlings were in decline when the interruption occurred, probably due to increasing mid-day environmental stress. In spite of irrigation, mid-day evaporative demand increased during mid-summer and resulted in reduced gas-exchange rates. Thus, interruption of ozone exposure may have had less impact on treatment differences than if a similar interruption had occurred in early spring.

### C. Lifestage variation in response to acidic rain and ozone exposure

A unique aspect of this study was the measurement of physiological and growth responses of genetically related seedlings and mature branches exposed to pollutants using common exposure devices in a common setting. As a result, we can make lifestage comparisons of performance and assess relative pollutant sensitivity.

A predominant hypothesis regarding ozone damage is that higher stomatal conductance leads to greater ozone damage (Miller *et al.* 1978, Coyne and Bingham 1982). Our mid-day gas exchange data do not support this hypothesis when comparing seedlings and mature branches. We observed  $g_s$  rates for one-year-old foliage that were similar for mature branches and seedlings. Yet, twice ambient ozone exposure resulted in significant declines in  $g_s$  of one-year-old foliage for mature branches but not for seedlings. Photosynthetic rates of one-year-old mature branches were significantly decreased for all genotypes while photosynthetic sensitivity to ozone was genotype dependant for older foliage of seedlings. Twice ambient ozone resulted in  $P_n$  declines for one-year-old foliage of 19 percent for mature branches and 14 percent for seedlings. For current-year foliage,  $g_s$  was greater for seedlings than for branches, yet reductions in  $P_n$  of current-year foliage was 9 percent for seedlings and 19 percent for mature branches.

Lifestage differences in ozone sensitivity are not easily discerned from the pigmentation and growth data. Chlorophyll a concentrations measured in September were decreased with 2xAMB ozone exposure for both seedlings and mature branches. However, ChlA tended to be approximately 30 percent less for seedling foliage regardless of ozone treatment. Significant ozone impact on growth was limited to diameter increment of mature branches.

Assessment of lifestage differences must be tempered by the conditions of the experiment. It must be noted that the entire seedling was exposed to the pollutant regime inside a BEC while only one branch of a tree was exposed. The impact of non-exposed tree components on branch response is not entirely known although previous work has indicated that branches are highly autonomous with respect to carbon movement (Houpis, unpublished data). Secondly, the trees were full-sibs while seedlings were half-sibs, thus, there was a greater degree of genotypic uniformity among replicate trees than among replicate seedlings.

### D. Genotypic variation in response to acidic rain and ozone exposure

Differences in ozone sensitivity among genotypes have been recognized for several species including loblolly pine (Sasek *et al.* 1991, Stow *et al.* 1991) and ponderosa pine (Temple *et al.* 1992). In this study,  $P_n$  rates for seedlings of clone 3088, regardless of foliage age class, were significantly lower, for plants exposed to 2xAMB ozone than for plants exposed to either CF or AMB ozone. Similar trends were not significant for seedlings of clone 3399 and absent for seedlings of clone 3087. It was also observed that early season ChlA, ChlB and Car concentrations for one-year-old foliage of mature branches were 20 to 30 percent lower for tissues exposed to 2xAMB ozone relative to tissue exposed to CF or AMB ozone. Pigment concentrations for similar foliage of clone

3088 branches exposed to 2xAMB ozone did not show a similar reduction, even though clone 3088 was apparently more ozone sensitive in terms of  $P_n$ . Among-family variation in ozone sensitivity has been attributed to variation among genotypes in ozone uptake and foliar morphology (Sasek *et al.* 1991, Coyne and Bingham 1981, Evans and Miller 1972). Although we did not examine foliar morphology, mid-day  $g_s$  estimates for clone 3087 were lowest throughout the season while those of clones 3088 and 3399 were indistinguishable. The low rates of  $g_s$  and negligible reduction in  $P_n$  under 2xAMB ozone for clone 3087 support the hypothesis that ozone sensitivity is dependent upon ozone uptake. The difference in  $P_n$  response to 2xAMB ozone between clones 3088 and 3399, in spite of similar  $g_s$ , suggests that differences in sensitivity may be related to factors other than potential ozone uptake, such as carbon allocation to repair or defense mechanisms.

#### E. Influence of acidic rain and ozone exposure on gas-exchange environmental response surfaces

Few studies have examined the effect of pollutant exposure on  $g_s$  and  $P_n$  response to microclimate. Response surfaces describing functional relationships between gas exchange parameters and independent microclimatic variable such as light intensity and temperature have been developed for several tree species (Hinkley *et al.* 1978, Running 1980, Livingston and Black 1987, Major 1990) including ponderosa pine (Rutter 1978, Anderson 1991). Coyne and Bingham (1982) is one of the few studies we know of which explicitly derived functional relationships between gas exchange variables and an independent environmental variable, light intensity, for trees exhibiting different degrees of pollutant injury.

The response surfaces derived in this study indicate that  $g_s$  and  $P_n$  response to temperature is impacted to a greater degree than the response to light intensity but the changes in the temperature response relationships were quite varied depending on pollutant treatment and genotype. It is difficult to provide a mechanistic explanation for the modification of  $P_n$  temperature response by ozone, but a highly speculative initial hypothesis is that temperature dependent enzyme activities may be influenced, possibly as a result of a loss in membrane integrity or due to changes in chloroplast or thylakoid structure. Changes in temperature response to acid rain exposure may possibly arise from changes in thermal energy balance as a result of modifications of the leaf cuticle. Although light intensity was more commonly a highly significant factor in accounting for  $P_n$  and  $g_s$  variation than was cuvette temperature, light response functions were relatively uniform among the response surfaces derived for the different treatments and genotypes. Most importantly, the  $P_n$  and  $g_s$  response surface analyses indicate that not only does pollutant exposure modify maximal rates of gas-exchange, but also that pollutant exposure may alter the relative response of  $P_n$  and  $g_s$  to various combinations of light and temperature. In other words, pollutant exposure may not only cause a downward shift in the response to light and temperature reflecting a change in  $P_n$  capacity, but may also cause a change in the form of the response relationships.

## **F. Future research needs**

Research to date has provided strong evidence indicating that many forest tree species will show some degree of injury response to long-term exposure to ozone. We have demonstrated that the injury response by ponderosa pine will be manifest as alterations in carbon assimilation rates, in foliar pigmentation, and in growth. The degree of injury response will vary with lifestage, foliage age-class and genotype.

Our data provide a basis for predicting physiological response at the tissue level and growth response at the individual seedling and branch level. Further research is required to integrate tissue and individual seedling or branch responses to stand and landscape levels. This will require the development of physiological process models that explicitly address pollutant impacts on basic plant processes. Future research should emphasize an understanding of the effects of ozone as an individual stress and as an interacting element with other edaphic and biotic stressors. Specific issues needing further research include:

- 1) Mesophyll and chloroplast mechanisms driving assimilation response to ozone exposure.
- 2) Lifestage influence on carbon allocation response to pollutant exposure.
- 3) Effect of pollutant exposure on carbon assimilate partitioning among structural and non-structural sugars, starch, and secondary carbon compounds such as lignins and terpenes that are increasingly being recognized as stress indicators.
- 4) Interaction effects of pollutants and edaphic stresses on physiological and growth performance.
- 5) Effects of stand composition and structure on microclimate and pollutant exposure.
- 6) Interspecific and intraspecific variation in physiological and growth responses to pollutant exposure.

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## Appendix A. Standard Operating Procedure for the Preparation of Acid Rain Solution

### 1. SCOPE and PURPOSE

The following standard operating procedure (SOP) is for the preparation of 3.0 pH and 5.1 pH acid precipitation solutions. The SOP was developed for the application of acid precipitation by spray nozzles within branch exposure chambers (BEC) at the Chico Air Pollution and Climate Change Research Facility.

### 2. MATERIALS

- A. Reverse osmosis water filtration system (Culligan, Inc., located adjacent to USFS injector room).
- B. Mixing Tanks. Four 750 liter mixing tanks.
- C. Brine catch tank. Mobile 1200 liter water tank.
- D. Distribution Carboys. Twenty 120 liter Nalgene carboys.
- E. Hand truck, located in the transformer shed.
- F. Acid pickup tubes. Twenty PVC pickup tubes inserted notch-down into carboy through small bung hole.
- G. Analytical scale (Mettler), located in Holt 245 (CSUC).
- H. Weighing paper.
- I. Scoopula.
- J. Ammonium Sulfate  $(\text{NH}_4)_2\text{SO}_4$ .
- K. Calcium Chloride Dihydrate  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ .
- L. Magnesium Nitrate  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ .
- M. Potassium Sulfate  $\text{K}_2\text{SO}_4$ .
- N. Sulfuric acid, concentrated (reagent grade).
- O. Nitric acid, concentrated (reagent grade).
- P. Deionized water.
- Q. pH buffers, 4.0 and 7.0 pH.
- R. Temperature Probe (Weston model 2261).
- S. pH Meter (Orion Research Ionalyzer model 407A).
- T. pH electrode (Ross).
- U. Beakers. Four 400 ml plastic beakers.
- V. Wash bottle.
- W. Graduated cylinders. One 250 ml and one 100 ml graduated cylinders.
- X. Pipette. One 10 ml pipette.
- Y. Pipette bulb.
- Z. Jars. Four 2 l plastic jars.

### 3. PROCEDURE

#### A. Deionized Water

1. Deionized water is produced using a Culligan, Inc. reverse osmosis water filtration system. Each of the four 800 l mixing tanks receives 450 l of deionized water.

2. The brine water produced during reverse osmosis is caught in a 1200 l mobile water tank. The brine water is dispersed on the surrounding gravel roads for evaporaton and dust abatement.
- B. Salts
1. Magnesium Nitrate is added to each tank of deionized water from stock solution (1384.2 mg/l) for a final concentration of 0.769 mg/l.
  2. Calcium Chloride is added to each tank of water from stock solution (1954.8 mg/l) for a final concentration of 1.086 mg/l.
  3. Ammonium Sulfate is added to each tank of water from stock solution (3330.0 mg/l) for a final concentration of 1.850 mg/l.
  4. Potassium Sulfate is added to each tank of water from stock solution (235.8 mg/l) for a final concentration of 0.131 mg/l.
- C. Acid Stock Solution
1. A 10M acid stock solution is made yielding a 2:3 ratio of equivalents of Sulfuric:Nitric acids, (1 liter 10M H<sub>2</sub>SO<sub>4</sub> = 20 equivalents, 3 liters 10M HNO<sub>3</sub> = 30 equivalents) therefore a 1:3 ratio of volumes (H<sub>2</sub>SO<sub>4</sub>:HNO<sub>3</sub>) is used.
  2. The acid stock solution bottle is kept in a secondary containment beaker.
- D. Acidifying Precipitation Solution
1. Calibrate the pH meter following all the manufacturer's instructions for initial calibration and warm-up.
  2. Mixing tanks #1 and #2 receive 10M acid stock solution dropwise while aerated until a pH of 3.0 is reached.
  3. The solutions in mixing tanks #3 and #4 are aerated for 10-15 min. and then the pH is recorded (approximately 5.1).
  4. Seventyfive liters of the resulting acid solutions are then distributed in carboys to their respective locations.



Table B1. Photosynthesis response surface models for mature branches and seedlings measured in August-September, 1992, by foliage age-class.

Photosynthesis Response Surface Models by Lifestage and Foliage Age-class: August-September, 1992				
Parameter	Mature Branches		Seedlings	
	Current-year	One-year-old	Current-year	One-year-old
Parameter Coefficients				
Intercept	-1.595	1.598	-2.784	-1.493
Light (L)	1.04E-2	6.95E-3	8.81E-3	2.84E-3
Air Temp. (T)	0.396	0.183	0.497	0.273
Vapor Press. (V)	-0.267	-0.438	-0.187	-0.043
L <sup>2</sup>	-5.71E-6	-4.15E-6	-5.58E-6	-1.97E-6
LxT	8.78E-5	6.77E-5	1.42E-4	2.50E-5
T <sup>2</sup>	-1.13E-2	-1.11E-2	-1.19E-2	-5.37E-3
LxV	-1.44E-4	-1.03E-4	-1.40E-4	9.02E-6
TxV	1.57E-2	3.50E-2	2.86E-3	8.58E-4
V <sup>2</sup>	-8.96E-3	-2.32E-2	5.32E-3	-1.98E-3
Model Order Significance (p>F)				
Linear	<0.001	<0.001	<0.001	<0.001
Quadratic	<0.001	0.001	<0.001	0.079
Cross-products	0.097	<0.001	0.080	0.893
Total Regression	<0.001	<0.001	<0.001	<0.001
Estimates of Model Fit and Precision				
S <sub>y<sup>2</sup></sub>	1.085	0.864	1.203	0.958
R <sup>2</sup>	0.622	0.485	0.537	0.161
Coeff. of Var.	20.00	23.83	23.15	37.97

Table B2. Photosynthesis response surface models for mature branches and seedlings measured in August-September, 1992, by ozone treatment.

Photosynthesis Response Surface Models by Lifestage and Ozone Treatment: August-September, 1992				
Parameter	Mature Branches		Seedlings	
	AMB	2xAMB	AMB	2xAMB
Parameter Coefficients				
Intercept	-2.569	1.641	-5.846	-0.439
Light (L)	9.27E-3	9.27E-3	6.94E-3	6.28E-3
Air Temp. (T)	0.318	0.198	0.541	0.303
Vapor Press. (V)	0.086	-0.554	0.235	-0.277
L <sup>2</sup>	-4.95E-6	-5.13E-6	-4.35E-6	-3.55E-6
LxT	1.09E-4	1.11E-4	8.97E-5	2.42E-4
T <sup>2</sup>	-6.28E-3	-8.11E-3	-8.14E-3	-7.80E-3
LxV	-2.16E-4	-2.97E-4	-7.66E-5	-5.46E-4
TxV	-3.52E-3	2.27E-2	-1.67E-2	2.34E-3
V <sup>2</sup>	3.45E-4	-5.70E-3	9.15E-3	-1.20E-2
Model Order Significance (p>F)				
Linear	<0.001	<0.001	<0.001	<0.001
Quadratic	0.006	0.013	0.012	0.197
Cross-products	0.218	0.005	0.367	0.012
Total Regression	<0.001	<0.001	<0.001	<0.001
Estimates of Model Fit and Precision				
S <sub>y<sup>2</sup></sub>	1.054	1.398	1.631	1.723
R <sup>2</sup>	0.581	0.414	0.284	0.252
Coeff. of Var.	21.90	33.03	38.12	49.08

Table B3. Stomatal conductance response surface models for mature branches measured in August-September, 1992, by genotype.

Response Surface Models for Mature Branch Stomatal Conductance by Genotype: August-September, 1992			
Parameter	Genotype 3087	Genotype 3088	Genotype 3399
Parameter Coefficients			
Intercept	0.215	0.027	0.370
Light (L)	4.00E-5	8.94E-5	-1.85E-4
Air Temp. (T)	3.45E-2	6.24E-3	1.13E-2
Vapor Press. (V)	-9.98E-2	-6.53E-3	-6.59E-2
L <sup>2</sup>	4.53E-9	-9.87E-8	7.32E-8
LxT	-3.81E-6	3.18E-6	5.16E-6
T <sup>2</sup>	-5.30E-4	5.43E-7	-2.11E-4
LxV	4.56E-6	-4.33E-6	-2.29E-6
TxV	8.91E-4	-2.32E-4	5.44E-4
V <sup>2</sup>	1.76E-3	6.12E-5	1.34E-3
Model Order Significance (p > F)			
Linear	<0.001	<0.001	<0.001
Quadratic	<0.001	0.542	<0.001
Cross-products	0.480	0.709	0.463
Total Regression	<0.001	<0.001	<0.001
Estimates of Model Fit and Precision			
S <sub>y<sup>2</sup></sub>	0.0562	0.0957	0.0666
R <sup>2</sup>	0.615	0.396	0.563
Coeff. of Var.	42.84	44.81	49.79

Table B4. Stomatal conductance response surface models for mature branches measured in August-September, 1992, by acid rain treatment.

Response Surface Models for Mature Branch Stomatal Conductance by Acid Rain Treatment: August-September, 1992		
Parameter	pH 5.1	pH 3.0
Parameter Coefficients		
Intercept	0.374	0.259
Light (L)	-1.27E-4	-7.10E-5
Air Temp. (T)	1.54E-2	8.92E-3
Vapor Press. (V)	-7.48E-2	-4.82E-2
L <sup>2</sup>	4.59E-8	-1.67E-8
LxT	2.42E-6	1.31E-6
T <sup>2</sup>	-2.68E-4	2.92E-6
LxV	-5.18E-8	6.70E-6
TxV	7.60E-4	-2.68E-4
V <sup>2</sup>	1.23E-3	1.54E-3
Model Order Significance (p>F)		
Linear	<0.001	<0.001
Quadratic	<0.001	<0.001
Cross-products	0.556	0.186
Total Regression	<0.001	<0.001
Estimates of Model Fit and Precision		
S <sub>y<sup>2</sup></sub>	0.0660	0.0543
R <sup>2</sup>	0.563	0.468
Coeff. of Var.	47.51	49.47

Table B5. Stomatal conductance response surface models for seedlings measured in August-September, 1992, by genotype and acid rain treatment.

Response Surface Models for Seedling Stomatal Conductance by Genotype and Acid Rain Treatment: August-September, 1992						
Parameter	Genotype 3087		Genotype 3088		Genotype 3399	
	pH 5.1	pH 3.0	pH 5.1	pH 3.0	pH 5.1	pH 3.0
Parameter Coefficients						
Intercept	-0.027	-0.083	0.292	-0.090	0.382	-0.090
Light (L)	-1.25E-5	-6.39E-5	-1.10E-4	-7.38E-6	1.23E-4	-1.33E-5
Air Temp. (T)	-3.61E-3	2.44E-2	-6.77E-4	2.81E-2	7.41E-3	2.30E-2
Vapor Press. (V)	2.31E-2	-2.22E-2	-3.41E-2	-2.45E-2	-8.11E-3	-1.58E-2
L <sup>2</sup>	3.90E-8	5.16E-8	6.46E-8	-1.09E-7	-1.52E-7	4.35E-8
LxT	4.55E-6	-3.91E-6	-5.48E-7	3.40E-7	1.67E-6	7.76E-6
T <sup>2</sup>	5.24E-4	-2.86E-5	-2.44E-4	-5.50E-4	-4.10E-4	1.08E-4
LxV	-1.20E-5	1.07E-5	4.44E-6	1.33E-5	5.50E-6	-2.20E-5
TxV	-1.96E-3	-1.35E-3	1.93E-3	4.96E-4	2.68E-3	-2.24E-3
V <sup>2</sup>	1.35E-3	1.72E-3	-1.37E-3	-3.53E-4	-6.44E-4	2.95E-3
Model Order Significance (p>F)						
Linear	0.020	0.005	0.170	0.002	<0.001	<0.001
Quadratic	0.676	0.071	0.983	0.294	0.053	0.021
Cross-products	0.168	0.692	0.242	0.412	0.052	0.016
Total Regression	0.064	0.156	0.394	0.012	<0.001	<0.001
Estimates of Model Fit and Precision						
S <sub>y<sup>2</sup></sub>	0.0977	0.0534	0.0562	0.0546	0.0758	0.0783
R <sup>2</sup>	0.231	0.283	0.157	0.281	0.499	0.501
Coeff. of Var.	57.09	58.23	51.89	45.51	43.35	61.18

Table B6. Photosynthesis response surface models for mature branches measured in November, 1992, by genotype and ozone treatment.

Response Surface Models for Mature Branch Photosynthesis by Genotype and Ozone Treatment: November, 1992						
Parameter	Genotype 3087		Genotype 3088		Genotype 3399	
	AMB	2xAMB	AMB	2xAMB	AMB	2xAMB
Parameter Coefficients						
Intercept	-2.336	-0.787	-5.350	-12.729	-1.243	0.824
Light (L)	1.01E-2	8.28E-3	8.15E-3	5.21E-3	1.06E-2	6.57E-3
Air Temp. (T)	0.501	0.313	0.818	1.825	0.402	-0.285
Vapor Press. (V)	-0.314	-7.98E-2	-0.479	-0.999	-0.272	0.579
L <sup>2</sup>	-6.09E-6	-6.38E-6	-5.54E-6	-3.10E-6	-6.91E-6	-2.69E-6
LxT	1.05E-4	1.83E-4	1.40E-4	-8.20E-5	1.34E-4	-5.42E-5
T <sup>2</sup>	-1.78E-2	-1.33E-2	-2.55E-2	-5.97E-2	-1.71E-2	1.68E-2
LxV	-1.03E-4	-1.23E-4	1.02E-4	-5.7E-5	-1.51E-4	1.35E-5
TxV	2.33E-2	1.39E-2	3.02E-2	6.97E-2	2.84E-2	-3.73E-2
V <sup>2</sup>	8.80E-3	-6.48E-3	1.07E-2	2.46E-2	1.53E-2	1.04E-2
Model Order Significance (p>F)						
Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic	0.008	<0.001	<0.001	0.624	0.064	0.415
Cross-products	0.744	0.442	0.018	0.911	0.569	0.556
Total Regression	<0.001	<0.001	<0.001	0.021	<0.001	0.009
Estimates of Model Fit and Precision						
S <sub>y</sub>	0.618	0.685	0.589	1.154	1.456	1.377
R <sup>2</sup>	0.838	0.716	0.830	0.501	0.484	0.354
Coeff. of Var.	12.88	14.83	13.83	32.32	30.39	44.60

Table B7. Photosynthesis response surfaces for seedlings measured in November, 1992, by genotype and ozone treatment.

Response Surface Models for Seedling Photosynthesis by Genotype and Ozone Treatment: November, 1992						
Parameter	Genotype 3087		Genotype 3088		Genotype 3399	
	AMB	2xAMB	AMB	2xAMB	AMB	2xAMB
Parameter Coefficients						
Intercept	-2.256	-1.673	-5.809	-2.782	-7.96	-4.499
Light (L)	9.28E-3	9.79E-3	1.16E-2	4.96E-3	1.21E-2	1.09E-2
Air Temp. (T)	0.645	0.162	1.16	0.539	0.966	-0.837
Vapor Press. (V)	-0.538	0.321	-0.872	-0.233	-0.146	-0.897
L <sup>2</sup>	-6.36E-6	-5.81E-6	-7.93E-6	-3.63E-5	-8.81E-6	-6.71E-6
LxT	1.58E-4	-5.13E-4	2.71E-5	-9.13E-5	7.17E-5	-1.64E-4
T <sup>2</sup>	-2.41E-3	-1.05E-3	-3.70E-2	-1.86E-2	-2.74E-2	-2.90E-2
LxV	-1.51E-4	-1.03E-4	8.51E-5	-6.67E-5	-1.71E-4	-2.76E-4
TxV	3.69E-2	1.84E-2	4.66E-2	2.07E-2	1.56E-2	5.08E-2
V <sup>2</sup>	-1.51E-2	-2.54E-3	1.42E-2	-8.88E-3	-8.00E-3	1.59E-2
Model Order Significance (p>F)						
Linear	<0.001	0.003	<0.001	<0.001	<0.001	<0.001
Quadratic	0.004	0.388	0.002	<0.001	0.004	0.064
Cross-products	0.480	0.905	0.241	0.112	0.372	0.413
Total Regression	<0.001	0.038	<0.001	<0.001	<0.001	<0.001
Estimates of Model Fit and Precision						
S <sub>y</sub>	0.540	1.251	0.683	0.235	0.660	1.220
R <sup>2</sup>	0.872	0.521	0.825	0.923	0.909	0.641
Coeff. of Var.	11.57	28.11	12.57	7.62	12.46	24.05

Table B8. Photosynthesis response surface models for mature branches measured in November, 1992, by genotype and foliage age-class.

Response Surface Models for Mature Branch Photosynthesis by Genotype and Foliage Age-class: November, 1992					
Parameter	Genotype 3087		Genotype 3088		Genotype 3399
	Current-year	One-year-old	Current-year	One-year-old	Current-year One-year-old
Parameter Coefficients					
Intercept	-1.511	0.617	-3.450	-5.331	-7.786 5.472
Light (L)	1.12E-2	9.48E-3	6.87E-2	6.41E-3	1.18E-2 5.60E-3
Air Temp. (T)	0.226	0.143	0.649	0.857	1.140 -0.469
Vapor Press. (V)	0.114	-0.149	-0.527	-0.476	-0.820 0.249
L <sup>2</sup>	-7.48E-6	-5.58E-6	-5.25E-6	-4.50E-6	-7.25E-6 -3.43E-6
LxT	1.16E-4	3.99E-5	2.08E-4	1.01E-4	1.67E-4 -7.20E-5
T <sup>2</sup>	-4.45E-3	-4.60E-3	-2.04E-2	-2.64E-2	-3.57E-2 1.62E-2
LxV	-5.09E-5	-6.47E-5	1.78E-4	-4.25-5	-2.37E-4 8.20E-5
TxV	-1.04E-2	6.26E-3	2.83E-2	3.05E-2	4.74E-2 -2.45E-2
V <sup>2</sup>	7.17E-3	-1.37E-3	9.74E-3	1.11E-2	-1.51E-2 1.03E-2
Model Order Significance (p>F)					
Linear	<0.001	<0.001	<0.001	<0.001	<0.001 0.005
Quadratic	<0.001	<0.001	0.186	0.011	0.115 0.774
Cross-products	0.271	0.807	0.419	0.187	0.315 0.638
Total Regression	<0.001	<0.001	<0.001	<0.001	<0.001 0.013
Estimates of Model Fit and Precision					
S <sub>y<sup>2</sup></sub>	0.410	0.502	1.048	0.548	1.454 1.500
R <sup>2</sup>	0.917	0.796	0.631	0.813	0.512 0.353
Coeff. of Var.	8.06	11.92	26.61	13.98	31.75 45.33

Table B9. Stomatal conductance response surface models for mature branches measured in November, 1992, by genotype.

Response Surface Models for Mature Branch Stomatal Conductance by Genotype: November, 1992			
Parameter	Genotype 3087	Genotype 3088	Genotype 3399
Parameter Coefficients			
Intercept	-0.089	-0.179	2.819
Light (L)	4.75E-5	4.33E-4	-3.11E-4
Air Temp. (T)	5.46E-2	4.79E-2	1.61E-2
Vapor Press. (V)	-6.67E-2	-7.03E-2	-5.19E-2
L <sup>2</sup>	-6.96E-8	-2.12E-7	1.15E-7
LxT	6.48E-6	4.32E-6	7.59E-6
T <sup>2</sup>	-2.29E-3	-1.18E-3	-4.93E-4
LxV	-7.06E-6	-2.81E-6	-2.53E-6
TxV	3.91E-3	2.05E-3	1.19E-3
V <sup>2</sup>	-1.16E-3	2.29E-6	2.23E-4
Model Order Significance (p>F)			
Linear	<0.001	<0.001	<0.001
Quadratic	0.001	<0.001	<0.001
Cross-products	0.004	0.141	0.080
Total Regression	<0.001	<0.001	<0.001
Estimates of Model Fit and Precision			
S <sub>y<sup>2</sup></sub>	0.0428	0.0832	0.0571
R <sup>2</sup>	0.478	0.484	0.561
Coeff. of Var.	46.34	64.02	49.32

Table B10. Stomatal conductance response surface models for mature branches measured in November, 1992, by acid rain treatment.

Response Surface Models for Mature Branch Stomatal Conductance by Acid Rain Treatment: November, 1992			
Parameter	pH 5.1	pH 3.0	
Parameter Coefficients			
Intercept	0.162	0.334	
Light (L)	1.07E-4	-8.41E-7	
Air Temp. (T)	1.16E-2	9.00E-3	
Vapor Press. (V)	-4.23E-2	-5.65E-2	
L <sup>2</sup>	-3.60E-8	-6.58E-8	
LxT	7.82E-9	5.57E-6	
T <sup>2</sup>	-2.35E-4	-3.65E-4	
LxV	-3.41E-6	-3.52E-6	
TxV	7.47E-4	1.42E-3	
V <sup>2</sup>	3.35E-4	9.93E-5	
Model Order Significance (p > F)			
Linear	<0.001	<0.001	
Quadratic	<0.001	<0.001	
Cross-products	0.342	0.369	
Total Regression	<0.001	<0.001	
Estimates of Model Fit and Precision			
S <sub>y<sup>2</sup></sub>	0.0607	0.0658	
R <sup>2</sup>	0.495	0.504	
Coeff. of Var.	58.01	53.91	

Table B11. Photosynthesis response surface models for seedlings measured in November, 1992, by genotype and acid rain treatment.

Response Surface Models for Seedling Photosynthesis by Genotype and Acid Rain Treatment: November, 1992						
Parameter	Genotype 3087		Genotype 3088		Genotype 3399	
	pH 5.1	pH 3.0	pH 5.1	pH 3.0	pH 5.1	pH 3.0
Parameter Coefficients						
Intercept	-1.817	-0.494	-10.525	-4.259	-4.155	-5.611
Light (L)	9.46E-2	1.18E-2	4.93E-3	6.80E-3	8.89E-2	1.27E-2
Air Temp. (T)	0.236	0.321	1.431	1.198	0.454	0.936
Vapor Press. (V)	0.246	-0.370	-0.570	-0.836	0.146	-0.730
L <sup>2</sup>	-5.48E-6	-6.70E-6	-3.70E-6	-6.28E-6	-5.95E-6	-8.31E-6
LxT	-2.84E-4	-3.07E-6	2.29E-4	2.59E-4	1.73E-4	1.11E-4
T <sup>2</sup>	2.64E-3	-1.11E-2	-6.10E-2	-6.25E-2	-1.10E-2	-3.02E-2
LxV	1.90E-5	-2.83E-5	-2.50E-4	-1.88E-4	-2.55E-4	-1.56E-5
TxV	-3.35E-2	2.25E-2	1.06E-1	1.03E-1	-5.18E-3	3.95E-2
V <sup>2</sup>	2.16E-2	-1.10E-2	-6.89E-2	4.86E-2	4.59E-3	-1.21E-2
Model Order Significance (p > F)						
Linear	0.004	<0.001	0.012	0.002	<0.001	<0.001
Quadratic	0.361	<0.001	0.253	0.036	0.170	0.002
Cross-products	0.989	0.110	0.549	0.303	0.727	0.503
Total Regression	0.049	<0.001	0.057	0.004	0.009	<0.001
Estimates of Model Fit and Precision						
S <sub>y<sup>2</sup></sub>	1.295	0.420	1.452	1.228	1.114	0.530
R <sup>2</sup>	0.477	0.921	0.443	0.560	0.569	0.940
Coeff. of Var.	27.37	9.52	34.39	28.82	26.31	10.15

Table B12. Stomatal conductance response surface models for seedlings measured in November, 1992, by genotype and acid rain treatment.

Response Surface Models for Seedling Stomatal Conductance by Genotype and Acid Rain Treatment: November, 1992						
Parameter	Genotype 3087		Genotype 3088		Genotype 3399	
	pH 5.1	pH 3.0	pH 5.1	pH 3.0	pH 5.1	pH 3.0
Parameter Coefficients						
Intercept	0.613	-0.318	0.016	0.350	0.451	0.558
Light (L)	-6.12E-6	-2.33E-4	5.28E-5	-7.00E-5	4.66E-5	2.48E-4
Air Temp. (T)	-5.34E-2	1.38E-1	7.59E-3	4.12E-2	-3.30E-2	-4.07E-2
Vapor Press. (V)	2.31E-2	-1.83E-1	-3.00E-3	-1.03E-1	1.76E-2	-2.41E-2
L <sup>2</sup>	5.52E-8	3.99E-8	-1.00E-7	-5.24E-8	1.37E-7	7.21E-8
LxT	-9.46E-6	7.70E-6	8.57E-6	7.42E-6	-6.47E-6	-1.96E-5
T <sup>2</sup>	2.56E-3	-4.17E-3	8.04E-5	-1.66E-3	1.59E-3	1.99E-3
LxV	1.26E-5	-1.72E-6	-8.25E-6	-4.21E-6	-4.93E-6	1.47E-5
TxV	-4.79E-3	6.84E-3	-8.87E-4	3.62E-3	-3.43E-3	-2.33E-3
V <sup>2</sup>	2.87E-3	-1.03E-3	7.76E-2	-3.58E-4	2.40E-3	1.91E-3
Model Order Significance (p > F)						
Linear	0.002	<0.001	0.002	<0.001	0.004	0.027
Quadratic	0.063	<0.001	0.346	<0.001	0.344	0.091
Cross-products	0.209	<0.001	0.671	0.366	0.213	0.846
Total Regression	0.005	<0.001	0.023	<0.001	0.019	0.074
Estimates of Model Fit and Precision						
S <sub>y<sup>2</sup></sub>	0.0345	0.0529	0.0384	0.0487	0.0655	0.0751
R <sup>2</sup>	0.598	0.901	0.496	0.870	0.532	0.601
Coeff. of Var.	28.87	29.46	36.29	31.44	40.08	60.22

